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(54) **ANTI-ACTIVIN A ANTIBODIES AND USES THEREOF**

(57) The disclosure provides compositions and methods relating to or derived from anti-activin A binding proteins, including antibodies. In particular embodiments, the disclosure provides fully human, humanized, and chimeric anti-activin A antibodies that bind human activin A, activin A-binding fragments and derivatives of such antibodies, and activin A-binding polypeptides comprising such fragments. Other embodiments provide nucleic acids encoding such antibodies, antibody fragments

and derivatives and polypeptides, cells comprising such polynucleotides, methods of making such antibodies, antibody fragments and derivatives and polypeptides, and methods of using such antibodies, antibody fragments and derivatives and polypeptides, including methods of treating or diagnosing subjects having activin A-related disorders or conditions including cachexia related to gonadal cancer, other cancers, rheumatoid arthritis, and other diseases.

Description

TECHNICAL FIELD

- 5 **[0001]** The present invention relates generally to cysteine knot domains of activin A and antigen binding agents capable of binding to activin A or fragments thereof.

BACKGROUND OF THE INVENTION

- 10 **[0002]** Many serious disease states are accompanied by a condition known as cachexia, which refers to loss of body cell mass. Body cell mass (BCM) consists of muscle mass, visceral mass and immune cell mass. BCM is the most active body component of the human body, counting ninety-five percent of all metabolic activity. A five percent loss of BCM leads to changed morbidity, loss of muscle strength, altered metabolism and increased risk of infection. A forty percent loss can result in death.

- 15 **[0003]** Examples of conditions in which cachexia plays a role in determining the outcome of the underlying disease cover a range of the major health problems today. In rheumatoid cachexia, rheumatoid arthritis (RA) patients lose thirteen to fifteen percent of BCM. Two-thirds of RA patients have cachexia, and this results in a two- to five-fold higher mortality. Other related conditions include rheumatoid cachectic obesity and hypercytokinaemic cachexia. Cancer-related cachexia contributes significantly to the morbidity and mortality, also affecting a patient's ability to tolerate potentially life-saving therapies.

- 20 **[0004]** Because of the common role of activin A in a number of widespread diseases, all of which have high rates of mortality, there is a long-felt need in the art for compositions and methods to prevent or reverse the disease-related cachexia. Such compositions and methods are provided herein.

25 BRIEF SUMMARY OF THE INVENTION

- [0005]** In one aspect, the present invention provides an isolated antigen binding protein comprising either: a. a light chain CDR3 comprising a sequence selected from the group consisting of: i. a light chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence selected from the group consisting of the light chain CDR3 sequences of L1-L14; ii. X₇₃ Q X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀; iii. L Q H N X₈₁ Y X₈₂ X₈₃ T; and iv. Q A W D X₈₄ S T X₈₅ X₈₆; b. a heavy chain CDR3 comprising a sequence selected from the group consisting of: i. a heavy chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence selected from the group consisting of the heavy chain CDR3 sequences of H1-H14; ii. X₈₇ X₈₈ X₈₉ X₉₀ X₉₁ X₉₂ X₉₃ X₉₄ F D Y; iii. X₉₅ X₉₆ X₉₇ Y X₉₈ D X₉₉ X₁₀₀ G W X₁₀₁ X₁₀₂ X₁₀₃; iv. X₁₀₄ X₁₀₅ X₁₀₆ X₁₀₇ X₁₀₈ X₁₀₉ Y X₁₁₀ X₁₁₁ X₁₁₂ X₁₁₃ X₁₁₄ X₁₁₅ X₁₁₆ X₁₁₇ X₁₁₈; or c. the light chain CDR3 sequence of (a) and the heavy chain CDR3 sequence of (b); wherein X₇₃ is a methionine residue, a glutamine residue, or an arginine residue, X₇₄ is an alanine residue, a tyrosine residue, a glutamine residue, or a serine residue, X₇₅ is a leucine residue, a tyrosine residue, or an asparagine residue, X₇₆ is a glutamine residue, a serine residue, or a threonine residue, X₇₇ is a threonine residue, a tyrosine residue, or an isoleucine residue, X₇₈ is a proline residue or a serine residue, X₇₉ is a cysteine residue, a tryptophan residue, a leucine residue, or a proline residue, X₈₀ is a serine residue or a threonine residue, X₈₁ is a threonine residue or a serine residue, X₈₂ is a proline residue or a threonine residue, X₈₃ is a phenylalanine residue or a tryptophan residue, X₈₄ is an arginine residue or a serine residue, X₈₅ is a valine residue or an alanine residue, X₈₆ is a valine residue or no residue, X₈₇ is a valine residue or no residue, X₈₈ is a glutamine residue or no residue, X₈₉ is an aspartate residue, a tryptophan residue, or no residue, X₉₀ is a serine residue, a leucine residue, or no residue, X₉₁ is an isoleucine residue, a glutamate residue, or a glutamine residue, X₉₂ is an alanine residue, a leucine residue, or a glycine residue, X₉₃ is an alanine residue or a leucine residue, X₉₄ is a proline residue, a tyrosine residue, or a glycine residue, X₉₅ is an aspartate residue or no residue, X₉₆ is a glutamine residue or no residue, X₉₇ is an aspartate residue or an alanine residue, X₉₈ is a tyrosine residue or a glycine residue, X₉₉ is a serine residue or a tyrosine residue, X₁₀₀ is a serine residue or an arginine residue, X₁₀₁ is a phenylalanine residue or no residue, X₁₀₂ is a glycine residue or an aspartate residue, X₁₀₃ is a histidine residue or a proline residue, X₁₀₄ is a glycine residue or no residue, X₁₀₅ is a serine residue, a glutamate residue, or no residue, X₁₀₆ is an arginine residue, a serine residue, or no residue, X₁₀₇ is an aspartate residue, an asparagine residue, a serine residue, or a glutamine residue, X₁₀₈ is a serine residue, an arginine residue, or a tryptophan residue, X₁₀₉ is a glycine residue, an aspartate residue, an asparagine residue, a tyrosine residue, or a leucine residue, X₁₁₀ is a serine residue, a glycine residue, an aspartate residue, or no residue, X₁₁₁ is a serine residue, a valine residue, an asparagine residue, or a tyrosine residue, X₁₁₂ is a serine residue, an asparagine residue, a tyrosine residue, or a histidine residue, X₁₁₃ is a tryptophan residue, a tyrosine residue, or a glutamine residue, X₁₁₄ is a histidine residue, an aspartate residue, a tyrosine residue, or no residue, X₁₁₅ is a phenylalanine residue, an alanine residue, or a glycine residue, X₁₁₆ an aspartate residue, a phenylalanine residue, a leucine

residue, or a methionine residue, X_{117} a tyrosine residue, or an aspartate residue, X_{118} is an isoleucine residue, a valine residue, or no residue, and the antigen binding protein binds specifically to human activin A.

[0006] In another aspect, the isolated antigen binding protein comprises an amino acid sequence selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of six amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of five amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0007] In a further aspect, the isolated antigen binding protein comprises an amino acid sequence selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of five amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence that differs by no more than a total of one amino acid addition, substitution, or deletion from a CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence that differs by no more than a total of one amino acid addition, substitution, or deletion from a CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0008] In a further aspect, the isolated antigen binding protein comprises an amino acid sequence selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of one amino acid addition, substitution, or deletion from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0009] In yet a further aspect, the isolated antigen binding protein comprises an amino acid sequence selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR3 sequence of L1-L14; c. a heavy chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and d. a heavy chain CDR3 sequence that differs by no more than a total of one amino acid addition, substitution, or deletion from a CDR3 sequence of H1-H14.

[0010] In another aspect, the isolated antigen binding protein comprises an amino acid sequence selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a heavy chain CDR2 sequence that differs by no more than a total of one amino acid addition, substitution, or deletion from a CDR2 sequence of H1-H14; and c. a heavy chain CDR3 sequence of H1-H14.

[0011] In a still further aspect, the isolated antigen binding protein comprises an amino acid sequence selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of one amino acid addition, substitution, or deletion from a CDR1 sequence of L1-L14; and b. a heavy chain CDR2 sequence of H1-H14.

[0012] In a yet further aspect, the isolated antigen binding protein comprises a CDR1 sequence of L1-L14.

[0013] The isolated antigen binding protein may comprise a sequence selected from the group consisting of: a. a light chain CDR1 sequence selected from the group consisting of: i. (R/K)SSQS(L/I)L(H/Y)S(T/S)(G/N)(Y/N)(N/K)(-K)YL(D/V); ii. RA(S/G)QGI(S/R)N(D/N)L(-V/G); iii. RASQISNYLNT; and iv. SG(D/E)K(L/W)G(D/E)K(F/Y)(A/V)(F/C); b. a light chain CDR2 sequence selected from the group consisting of: i. (H/Q/L)D(T/N/S)KRPS; and ii. $X_{40} X_{41} S X_{42} X_{43} X_{44} S$, wherein X_{40} is an alanine residue, a tryptophan residue, or a leucine residue, X_{41} is a threonine residue, an alanine residue, or a glycine residue, X_{42} is a serine residue, a methionine residue, or a phenylalanine residue, X_{43} is a leucine residue or an arginine residue, X_{44} is a glutamine residue, a glutamate residue, or an alanine residue, c. a heavy chain CDR1 sequence selected from the group consisting of: i. GGS(I/F)(N/S)(S/A)(-G)(-G)(F/Y)YWS; ii. $G X_{27} X_{28} F X_{29} X_{30} Y X_{31} X_{32} X_{33}$, wherein X_{27} is a tyrosine residue or a phenylalanine residue, X_{28} is a threonine residue or a serine residue, X_{29} is a threonine residue, a serine residue, or an isoleucine residue, X_{30} is a glycine residue or a serine residue, X_{31} is a tyrosine residue, a glycine residue, or a tryptophan residue, X_{32} is an isoleucine residue or a methionine residue, X_{33} is a histidine residue or a glycine residue; and iii. G(Y/F)TF(T/S)(-S/A)Y(G/W)(L/M/I)(S/H); d. a heavy chain CDR2 sequence selected from the group consisting of: i.

(Y/E)I(S/Y/N)(Y/H)SG(S/G)T(Y/N)YNPSLK(S/R); ii. (V/N)I(K/W)(Y/Q)DGS(N/E/T)-(K/E)Y(H/Y)(A/V)DSVKG; and iii. X₆₀ I X₆₁ X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ T X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂ Q G, wherein X₆₀ is a tryptophan residue or an isoleucine residue, X₆₁ is an asparagine residue, an isoleucine residue, a serine residue, or a tyrosine residue, X₆₂ is a proline residue or an alanine residue, X₆₃ is an asparagine residue, a tyrosine residue, or a glycine residue, X₆₄ is a serine residue, an asparagine residue, or an aspartate residue, X₆₅ is a glycine residue or a serine residue, X₆₆ is a glycine residue, an asparagine residue, or an aspartate residue, X₆₇ is an asparagine residue or an arginine residue, X₆₈ is a tyrosine residue or a serine residue, X₆₉ is an alanine residue or a serine residue, X₇₀ is a glutamine residue or a proline residue, X₇₁ is a lysine residue or a serine residue, and X₇₂ is a phenylalanine residue or a leucine residue, wherein amino acid residue symbols enclosed in parentheses identify alternative residues for the same position in a sequence.

[0014] In certain aspects, the isolated antigen binding protein comprises a heavy chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0015] In further aspects, the isolated antigen binding protein comprises a heavy chain CDR3 sequence that differs by no more than a total of one amino acid addition, substitution, or deletion from a CDR3 sequence of H1-H14.

[0016] In yet further aspects, the isolated antigen binding protein comprises a heavy chain CDR3 sequence of H1-H14.

[0017] In another aspect, the isolated antigen binding protein comprises two amino acid sequences selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of six amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of five amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0018] In a further aspect, the isolated antigen binding protein comprises three amino acid sequences selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of six amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of five amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0019] In another aspect, the isolated antigen binding protein comprises four amino acid sequences selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of six amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of five amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0020] In another aspect, the isolated antigen binding protein comprises five amino acid sequences selected from the group consisting of: a. a light chain CDR1 sequence that differs by no more than a total of six amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of five amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0021] In a still further aspect, the isolated antigen binding protein comprises :a. a light chain CDR1 sequence that differs by no more than a total of six amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14; b. a light chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of L1-L14; c. a light chain CDR3 sequence that differs by no more than a total of three

amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14; d. a heavy chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of H1-H14; e. a heavy chain CDR2 sequence that differs by no more than a total of five amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14; and f. a heavy chain CDR3 sequence that differs by no more than a total of four amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14.

[0022] In another aspect, the isolated antigen binding protein comprises either: a. a light chain variable domain comprising: i. a light chain CDR1 sequence; ii. a light chain CDR2 sequence; and iii. a light chain CDR3 sequence; b. a heavy chain variable domain comprising: i. a heavy chain CDR1 sequence; ii. a heavy chain CDR2 sequence; and iii. a heavy chain CDR3 sequence; or c. the light chain variable domain of (a) and the heavy chain variable domain of (b).

[0023] In one embodiment, the isolated antigen binding protein comprises a combination of a light chain variable domain and a heavy chain variable domain selected from the group of combinations consisting of: L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14.

[0024] In one embodiment, the isolated antigen binding protein further comprises: the kappa light chain constant sequence of SEQ ID NO:84, 100 or 108, and/or the heavy chain constant sequence of SEQ ID NO:214, 215 or 221.

[0025] In one embodiment, the isolated antigen binding protein, when bound to activin A: a. inhibits activin A; b. cross-competes with a reference antibody for binding to activin A; c. binds to the same epitope of activin A as said reference antibody; d. binds to activin A with substantially the same K_d as said reference antibody; or e. binds to activin A with substantially the same off rate as said reference antibody; wherein the reference antibody comprises a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14.

[0026] In one embodiment, the isolated antigen binding protein, when bound to a human activin A, inhibits binding of activin A to human activin A receptor; attenuates cachexia in colon tumor-bearing mice; ameliorates the loss of body weight in colon tumor-bearing mice; ameliorates the loss of body weight in a collagen-induced animal model of rheumatoid arthritis; ameliorates the loss of muscle mass in a collagen-induced animal model of rheumatoid arthritis; ameliorates the loss of fat mass in a collagen-induced animal model of rheumatoid arthritis; and/or ameliorates the loss of body weight in a AAV-activin A transfected animal model.

[0027] In one aspect, the isolated antigen binding protein comprises: a. a human antibody; b. a humanized antibody; c. a chimeric antibody; d. a monoclonal antibody; e. a polyclonal antibody; f. a recombinant antibody; g. an antigen-binding antibody fragment; h. a single chain antibody; i. a diabody; j. a triabody; k. a tetrabody; l. a Fab fragment; a F(ab')₂ fragment; n. a domain antibody; o. an IgD antibody; p. an IgE antibody; q. an IgM antibody; r. an IgG1 antibody; s. an IgG2 antibody; t. an IgG3 antibody; u. an IgG4 antibody; or v. an IgG4 antibody having at least one mutation in a hinge region that alleviates a tendency to form intra-H chain disulfide bond.

[0028] Also provided is a human antigen binding protein specific for activin A, wherein the antigen binding protein possesses at least one *in vivo* biological activity of a human anti-activin A antibody; such as the attenuation of cachexia.

[0029] Further provided is a human antigen binding protein that ameliorates the loss of body weight in colon tumor-bearing mice, or that ameliorates the loss of body weight in a collagen-induced animal model of rheumatoid arthritis.

[0030] Also provided is a human antigen binding protein that ameliorates the loss of muscle mass in a collagen-induced animal model of rheumatoid arthritis, that ameliorates the loss of fat mass in a collagen-induced animal model of rheumatoid arthritis or that ameliorates the loss of body weight in a AAV-activin A transfected animal model.

[0031] Further provided is a human antigen binding protein specific for activin A, wherein the antigen binding protein inhibits the binding of activin A to activin A receptor *in vitro*.

[0032] Also provided is a human antigen binding protein specific for activin A, wherein the antigen binding protein inhibits the binding of activin A to activin A receptor *in vivo*.

[0033] In another aspect, provided is an isolated polynucleotide comprising a sequence that encodes the light chain, the heavy chain, or both of an antigen binding protein of the invention; the polynucleotide may comprise a light chain variable domain nucleic acid sequence of SEQ ID NO:1, 17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193 or 209 and/or a heavy chain variable domain nucleic acid sequence of SEQ ID NO:2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194 or 210.

[0034] Also provided is a plasmid comprising the isolated polynucleotide; the plasmid may be an expression vector; and an isolated cell is provided that comprises the polynucleotide; the isolated cell may be a hybridoma, and the cell may be a CHO cell.

[0035] Further provided is a method of making an antigen binding protein that binds human activin A, comprising incubating the isolated cell under conditions that allow it to express said antigen binding protein.

[0036] Also provided is a pharmaceutical composition comprising the antigen binding protein of the invention, a method of treating a condition in a subject comprising administering to the subject the pharmaceutical composition, wherein the condition is treatable by reducing the activity of activin A in said subject; the subject may be a human being, and the condition may be cachexia associated with a tumor, wherein the tumor is a gonadal tumor, such as ovarian cancer, benign prostatic hyperplasia, prostate intraepithelial neoplasia, or prostate cancer, or wherein the tumor is bladder

cancer, Wilm's tumor, pancreatic cancer, breast cancer, bone cancer, lung cancer, colorectal cancer, cervical cancer, synovial sarcoma, vasoactive intestinal peptide secreting tumors, glioblastoma, medulloblastoma, head and neck squamous cell cancer, oral cancer, oral leukoplakia, , anal cancer, esophageal cancer, gastric cancer, bone cancer, or metastatic cancer; the condition may be cachexia associated with a rheumatoid arthritis; or the condition may be the

need for decreasing activin A activity in a subject.

[0037] In another aspect, the present invention provides a method of maintaining muscle mass of a subject comprising administering to said subject said pharmaceutical composition.

[0038] In another aspect, the present invention provides a method of decreasing activin A activity in a subject in need thereof comprising administering to said subject said pharmaceutical composition.

[0039] In another aspect, the present invention provides antibodies that are able to specifically bind amino acids K13-Y39 of activin A *in vitro* or *in vivo*. In another aspect, the present invention provides antibodies that are able to specifically bind amino acids V82-N107 *in vitro* or *in vivo*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040]

Figure 1 provides the muscle mass change for collagen induced arthritis mice treated with anti-activin A antibody A1.

Figure 2 provides the fat mass change for collagen induced arthritis mice treated with anti-activin A antibody A1.

Figure 3 provides data showing that anti-activin A treatment using antibodies A1, A2 and A3 prevents body weight loss in young adult nude mice with an intramuscular CHO/Activin xenograft.

Figure 4 provides NMR data showing that anti-activin A treatment prevents loss of lean body mass in young adult nude mice with an intramuscular CHO/Activin xenograft.

Figure 5 provides the effect of anti-activin A antibody A1 on body weight changes in AAV-activin A transduced mice.

Figure 6 provides the gastrocnemius muscle mass in a CDF1 mouse Colon-26 cancer cachexis model with and without treatment with anti-activin A antibody A1, eighteen days after tumor inoculation.

Figure 7 shows a model of activin A, with the region of antibody binding circled. K21, K103 and X94 refer to lysine residues at position 21 and 103, and a tyrosine residue at position 94.

Figure 8 is a graph showing the binding affinities of antibodies A1, A2 and A3 as determined using KinExA. The dissociation equilibrium constant was obtained from nonlinear regression of the competition curves using a dual-curve one-site homogeneous binding model using the KinExA software.

Figure 9 shows epitope regions that were not protected from degradation by binding of antibodies A1, A2 or A3.

Figure 10 is a graph showing binding affinities of antibodies A1, A2, and A3 for intact activin A (indicated by lot 55), as well as activin A that is cleaved at the tyrosine residue at amino acid position number 94 (indicated by lot 38).

Figure 11 is a graph showing binding affinities of antibodies A1, A2, and A3, as well as two commercially available antibodies for activin A or activin B on immobilized antibody surfaces.

Figure 12 shows antibody binding to activin A/activin B chimeras by antibody A1 and A2, as well as two commercially available activin A antibodies.

Figure 13 shows the amino acid sequences of activin A/activin B chimeras utilized in the antibody testing described in Figure 11.

Figure 14 shows binding of several antibodies, including A1, A2, and A3, to different epitopes of activin A; two commercially available activin A antibodies were also tested.

DETAILED DESCRIPTION

[0041] The present invention relates to regions of the human activin A that contain cysteine knot domains recognized by antibodies that also bind to full-length activin A, and/or a region of activin A that overlaps or encompasses a cysteine knot region of activin A, and methods of making and using these cysteine knot domains. The invention also provides antigen binding agents, including antibodies, that specifically bind to activin A or portions of activin A, and methods for using such binding agents. The binding agents are useful to block or impair binding of human activin A to one or more ligand.

[0042] Mortality from congestive heart failure (CHF) is related to cachexia. In one study (Anker, S. D. and Coats, A. J., Chest 115:836-847, 1999), sixteen percent of an unselected CHF outpatient population was cachectic. This state was predictive of impaired prognosis independent of age, functional disease classification, left ventricular ejection fraction, and peak oxygen consumption. The mortality in the cachectic cohort was fifty percent at eighteen months.

[0043] One pathway common to the disease progression in cancer, rheumatoid arthritis, chronic renal failure, congestive heart failure, and other conditions in which cachexia is a factor is the activin A pathway. Muscle wasting and weakness are common in many disease states and conditions including aging, cancer cachexia, sepsis, denervation,

disuse, inactivity, burns, HIV-acquired immunodeficiency syndrome (AIDS), chronic kidney or heart failure, unloading/microgravity, and muscular dystrophies. Activins and inhibins are members of the TGF-beta superfamily. Activins and inhibins function as stimulators and inhibitors, respectively, of pituitary follicle-stimulating hormone (FSH) secretion and biosynthesis. Activin A is the predominant form of activin. In reproductive biology, activins and inhibins are important

regulators of the ovarian cycle and the ovulation process, and may play a role in embryo implantation, and/or maintenance of pregnancy. (O'Connor et al., Human Reproduction, V. 14, No. 3, 827-832, March 1999; Draper et al., Endocrin., V. 138, No. 7: 3042-3046; Jones, et al., Endocrin. V. 147, No. 2: 724-732, Feb. 2006). The identification of inhibins and activins in a wide variety of tissues suggests that these factors play much greater roles than the control of FSH secretion.

[0044] Activins interact with two structurally related classes of serine/threonine kinase receptors (type I and type II).

Inhibin antagonizes activin by binding to the proteoglycan, betaglycan, and forming a stable complex with and thereby sequestering type II activin receptors while excluding type I receptors. Two major forms of activin exist: activin A is a homodimer of β_A -subunits and activin B is a homodimer of β_B -subunits. (Vale, et al., Recent Prog Horm Res V. 44: 1-34, 1988). Heterodimers of an α -subunit that is dissimilar to either β -subunit results in the functional antagonist inhibin.

[0045] The literature has shown that activin A is overexpressed and/or localized in cancer tissues. For example, high levels of serum activin A were found in women with endometrial and cervical carcinoma (Petraglia, F. et al., Jour. Clin. Endocrin. Metab. 83:1194-1200, 1998). Activin A was overexpressed in stage IV colorectal cancer (Wildi, S. et al., Gut 49:409-417, 2001). A role of activin A in ovarian cancer was reported (Steller, M.D. et al., Mol. Cancer Res. 3:50-61, 2005).

[0046] The literature has also implicated activin A in renal disease. (Yamashita, S. et al. J. Am. Soc. Nephrol. 15:91-101, 2004.) Serum immunoreactive activin A levels in normal subjects and patients with disease were reported by Harada, K. et al. in J. Clin. Endocrin. and Metab. 81:2125-2130, 1996. Activin A is a potent activator of renal interstitial fibroblasts (Harada, K. et al., J. Am. Soc. Nephrol. 15:91-101, 2004). Glomerular activin A overexpression is linked to fibrosis in anti-Thy 1 glomerulonephritis (Gaedeke, J. et al., Neph. Dial. Transpl. 20:319-328, 2005).

[0047] Serum activin A levels in heart failure patients increased according to disease severity (Yndestad et al., Circulation 109:1379-1385, 2004). In a rat model of heart failure, serum activin A elevated immediately after myocardial infarct and persisted for six months, and activin A immunostaining was localized solely to cardiomyocytes (Yndestad et al., 2004). Elevated levels of activin A were reported in heart failure (Yndestad, A. et al., Circulation 109:1379-1385, 2004).

[0048] The present invention provides compositions, kits, and methods relating to molecules that bind to the activin A, including molecules that agonize or antagonize activin A, such as anti-activin A antibodies, antibody fragments, and antibody derivatives, e.g., antagonistic anti-activin A antibodies, antibody fragments, or antibody derivatives. Also provided are compositions, kits, and methods relating to molecules that specifically bind to a portion of activin A, such as amino acids R13-Y39, or amino acids V82-N107 of activin A. Such molecules may include antibodies, antibody fragments, and antibody derivatives. Also provided are nucleic acids, and derivatives and fragments thereof, comprising a sequence of nucleotides that encodes all or a portion of a polypeptide that binds to activin A, e.g., a nucleic acid encoding all or part of an anti-activin A antibody, antibody fragment, antibody variant, or antibody derivative, plasmids and vectors comprising such nucleic acids, and cells or cell lines comprising such nucleic acids and/or vectors and plasmids. The provided methods include, for example, methods of making, identifying, or isolating molecules that bind to activin A, such as anti-activin A antibodies, methods of determining whether a molecule binds to activin A, methods of making compositions, such as pharmaceutical compositions, comprising a molecule that binds to activin A, and methods for administering a molecule that binds activin A to a subject, for example, methods for treating a condition mediated by activin A, and for modulating a biological activity of activin A *in vivo* or *in vitro*.

[0049] Polynucleotide and polypeptide sequences are indicated using standard one- or three-letter abbreviations. Unless otherwise indicated, polypeptide sequences have their amino termini at the left and their carboxy termini at the right, and single-stranded nucleic acid sequences, and the top strand of double-stranded nucleic acid sequences, have their 5' termini at the left and their 3' termini at the right. A particular polypeptide or polynucleotide sequence also can be described by explaining how it differs from a reference sequence. Unless otherwise indicated, it is understood that polynucleotide and polypeptide sequences include each nucleic acid or amino acid listed, respectively, as well as the intervening nucleic acids or amino acids. For example, the polypeptide sequence R13-Y39 sets forth a polypeptide sequence that includes the amino acids R13, and Y39, as well as the amino acids found between R13 and Y39 in the polypeptide sequence. Correspondingly, the polynucleotide sequence C1-T5 sets forth a polynucleotide sequence that includes nucleic acids C1, and T5, as well as nucleic acids at positions 2, 3, and 4 of the sequence. Accordingly, designations of SEQ ID NO: 1-5 likewise designates the inclusive group of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5. Finally, amino acid groupings are also intended to be inclusive, unless otherwise designated. For example, the phrase "amino acids 1-5 of SEQ ID NO: 28" includes amino acids at positions 1, 2, 3, 4, and 5 of SEQ ID NO: 28.

[0050] Polynucleotide and polypeptide sequences of particular light and heavy chain variable domains are shown below. Antibodies comprising a light chain and heavy chain are designated by combining the name of the light chain and the name of the heavy chain variable domains. For example, "L4H7," indicates an antibody comprising the light chain variable domain of L4 and the heavy chain variable domain of H7.

[0051] Kappa light chain constant sequences are shown in SEQ ID NO:84, 100 and 108, and heavy chain constant sequence are shown in SEQ ID NO:214, 215 and 221. Polynucleotides encoding these sequences are shown in, for the light chains, respectively, SEQ ID NO:222, 223 and 239, and for the heavy chains, respectively, SEQ ID NO:240, 241, and 242. Thus, in addition to the variable sequences as disclosed herein, an antibody can comprise one or both of SEQ ID NO:84 and 214; or SEQ ID NO:215 and 223; or SEQ ID NO:108 and 221.

[0052] In other embodiments, an antibody may comprise a specific heavy or light chain, while the complementary light or heavy chain variable domain remains unspecified. In particular, certain embodiments herein include antibodies that bind a specific antigen (such as activin A) by way of a specific light or heavy chain, such that the complementary heavy or light chain may be promiscuous, or even irrelevant, but may be determined by, for example, screening combinatorial libraries. Portolano et al., J. Immunol. V. 150 (3), pp. 880-887 (1993); Clackson et al., Nature v. 352 pp. 624-628 (1991).

[0053] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0054] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

The term "isolated molecule" (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or synthesized in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[0055] The terms "activin A inhibitor" and "activin A antagonist" are used interchangeably. Each is a molecule that detectably inhibits at least one function of activin A. Conversely, an "activin A agonist" is a molecule that detectably increases at least one function of activin A. The inhibition caused by an activin A inhibitor need not be complete so long as it is detectable using an assay. Any assay of a function of activin A can be used, examples of which are provided herein. Examples of functions of activin A that can be inhibited by an activin A inhibitor, or increased by an activin A agonist, include binding to activin A. Examples of types of activin A inhibitors and activin A agonists include, but are not limited to, activin A binding polypeptides such as antigen binding proteins (e.g., activin A inhibiting antigen binding proteins), antibodies, antibody fragments, and antibody derivatives.

[0056] The terms "peptide," "polypeptide" and "protein" each refers to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, e.g., native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins. A peptide, polypeptide, or protein may be monomeric or polymeric.

[0057] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to a corresponding full-length protein. Fragments can be, for example, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 50, 70, 80, 90, 100, 150 or 200 amino acids in length. Fragments can also be, for example, at most 1,000, 750, 500, 250, 200, 175, 150, 125, 100, 90, 80, 70, 60, 50, 40, 30, 20, 15, 14, 13, 12, 11, or 10 amino acids in length. A fragment can further comprise, at either or both of its ends, one or more additional amino acids, for example, a sequence of amino acids from a different naturally-occurring protein (e.g., an Fc or leucine zipper

domain) or an artificial amino acid sequence (e.g., an artificial linker sequence).

[0058] Polypeptides of the invention include polypeptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties. Analogs include muteins of a polypeptide. For example, single or multiple amino acid substitutions (e.g., conservative amino acid substitutions) may be made in the naturally occurring sequence (e.g., in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A "conservative amino acid substitution" is one that does not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterize the parent sequence or are necessary for its functionality). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991), which are each incorporated herein by reference.

[0059] A "variant" of a polypeptide (e.g., an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to the native polypeptide sequence, and retains essentially the same biological activity as the native polypeptide. The biological activity of the polypeptide can be measured using standard techniques in the art (for example, if the variant is an antibody, its activity may be tested by binding assays, as described herein). Variants of the invention include fragments, analogs, recombinant polypeptides, synthetic polypeptides, and/or fusion proteins. A "derivative" of a polypeptide is a polypeptide (e.g., an antibody) that has been chemically modified, e.g., via conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (e.g., human serum albumin), phosphorylation, and glycosylation. Unless otherwise indicated, the term "antibody" includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

[0060] An "antigen binding protein" is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (e.g., an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

[0061] An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

[0062] Naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in *Sequences of Proteins of Immunological Interest*, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991.

[0063] An "antibody" refers to an intact immunoglobulin or to an antigen binding portion thereof that competes with the intact antibody for specific binding, unless otherwise specified. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, *inter alia*, Fab, Fab', F(ab')₂, Fv, domain antibodies (dAbs), and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least

a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

[0064] A Fab fragment is a monovalent fragment having the V_L , V_H , C_L and C_{H1} domains; a $F(ab')_2$ fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the V_H and C_{H1} domains; an Fv fragment has the V_L and V_H domains of a single arm of an antibody; and a dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or V_L domain (US Pat. No. 6,846,634, 6,696,245, US App. Pub. No. 05/0202512, 04/0202995, 04/0038291, 04/0009507, 03/0039958, Ward et al., Nature 341:544-546, 1989).

[0065] A single-chain antibody (scFv) is an antibody in which a V_L and a V_H region are joined via a linker (e.g., a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (see, e.g., Bird et al., 1988, Science 242:423-26 and Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-83). Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90:6444-48, and Poljak et al., 1994, Structure 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

[0066] Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat *et al.* in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. An antigen binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

[0067] An antigen binding protein may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For example, a naturally occurring human immunoglobulin typically has two identical binding sites, while a "bispecific" or "bifunctional" antibody has two different binding sites.

[0068] The term "human antibody," also referred to as "fully human antibody," includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of ways, examples of which are described below, including through the immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes.

[0069] A humanized antibody has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

[0070] The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-activin A antibody. In another embodiment, all of the CDRs are derived from a human anti-activin A antibody. In another embodiment, the CDRs from more than one human anti-activin A antibodies are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-activin A antibody, a CDR2 and a CDR3 from the light chain of a second human anti-activin A antibody, and the CDRs from the heavy chain from a third anti-activin A antibody. Further, the framework regions may be derived from one of the same anti-activin A antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or light chain is identical with, homologous to, or derived from an antibody from a particular species or belonging to a particular antibody class or subclass, while

the remainder of the chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies that exhibit the desired biological activity (*i.e.*, the ability to specifically bind activin A).

[0071] Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification and using techniques well-known in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See, *e.g.*, Bowie et al., 1991, *Science* 253:164.

[0072] Additionally, antigen specific (*i.e.* activin A specific) antibodies can be produced by methods known in the art by using a specific VL or VH domain to screen a library of the complementary variable domain. Such methods of producing antibodies are known in the art. For example, antibody fragments fused to another protein, such as a minor coat protein, can be used to enrich phage with antigen. Then, using a random combinatorial library of rearranged heavy (VH) and light (VL) chains from mice immune to the antigen (*e.g.* activin A), diverse libraries of antibody fragments are displayed on the surface of the phage. These libraries can be screened for complementary variable domains, and the domains purified by, for example, affinity column. See Clackson et al., *Nature*, V. 352 pp. 624-628 (1991).

[0073] In another example, individual VL or VH chains from an antibody (*i.e.* activin A antibody) can be used to search for other VH or VL chains that could form antigen-binding fragments (or Fab), with the same specificity. Thus, random combinations of VH and VL chain Ig genes can be expressed as antigen-binding fragments in a bacteriophage library (such as fd or lambda phage). For instance, a combinatorial library may be generated by utilizing the parent VL or VH chain library combined with antigen-binding specific VL or VH chain libraries, respectively. The combinatorial libraries may then be screened by conventional techniques, for example by using radioactively labeled probe (such as radioactively labeled activin A). See, for example, Portolano et al., *J. Immunol.* V. 150 (3) pp. 880-887 (1993).

[0074] A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a particular species or isotype and the framework of another antibody of the same or different species or isotype.

[0075] A "multi-specific antibody" is an antibody that recognizes more than one epitope on one or more antigens. A subclass of this type of antibody is a "bi-specific antibody" which recognizes two distinct epitopes on the same or different antigens.

[0076] An "antigen binding domain," "antigen binding region," or "antigen binding site" is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

[0077] An "epitope" is the portion of a molecule that is bound by an antigen binding protein (*e.g.*, by an antibody). An epitope can comprise non-contiguous portions of the molecule (*e.g.*, in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein), and includes the end sequence amino acids listed. For example the polypeptide sequence R13-Y39 includes amino acids R13, and Y39, as well as the amino acids found between R13 and Y39 in the sequence. In embodiments in which the epitope comprises non-contiguous portions of a molecule, the sequences will be noted accordingly.

[0078] The "percent identity" of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, CA)) using its default parameters.

[0079] The terms "polynucleotide," "oligonucleotide" and "nucleic acid" are used interchangeably throughout and include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs (*e.g.*, peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding an antibody, or a fragment, derivative, mutein, or variant thereof, of the invention.

[0080] Two single-stranded polynucleotides are "the complement" of each other if their sequences can be aligned in an anti-parallel orientation such that every nucleotide in one polynucleotide is opposite its complementary nucleotide in the other polynucleotide, without the introduction of gaps, and without unpaired nucleotides at the 5' or the 3' end of either sequence. A polynucleotide is "complementary" to another polynucleotide if the two polynucleotides can hybridize to one another under moderately stringent conditions. Thus, a polynucleotide can be complementary to another polynucleotide without being its complement.

[0081] A "vector" is a nucleic acid that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (*e.g.*, replication defective retroviruses, adenoviruses

and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An "expression vector" is a type of vector that can direct the expression of a chosen polynucleotide.

[0082] A nucleotide sequence is "operably linked" to a regulatory sequence if the regulatory sequence affects the expression (e.g., the level, timing, or location of expression) of the nucleotide sequence. A "regulatory sequence" is a nucleic acid that affects the expression (e.g., the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (e.g., polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA and Baron et al., 1995, *Nucleic Acids Res.* 23:3605-06.

[0083] A "host cell" is a cell that can be used to express a nucleic acid, e.g., a nucleic acid of the invention. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Example 3 herein described the use of CS-9 cells. Examples of other host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman et al., 1981, *Cell* 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen et al., 1998, *Cytotechnology* 28:31), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMahan et al., 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Antigen binding proteins

[0084] In one aspect, the present invention provides antigen binding proteins (e.g., antibodies, antibody fragments, antibody derivatives, antibody muteins, and antibody variants), that bind to activin A, e.g., human activin A.

[0085] Antigen binding proteins in accordance with the present invention include antigen binding proteins that inhibit a biological activity of activin A. For example, antigen binding proteins may attenuate cachexia, and this activity can be present when the antigen binding protein is fully human, such as a fully human antibody.

[0086] Different antigen binding proteins may bind to different domains or cysteine knot domains of activin A or act by different mechanisms of action. Examples include but are not limited to antigen binding proteins that specifically bind one or more particular cysteine knot domains, or regions interspersed between disulfide bonds, including regions spanning from about amino acids 4-12, amino acids 11-81, amino acids 11-33, amino acids 13-39, amino acids 40-113, amino acids 44-115, amino acids 81-111, and/or amino acids 82-107 of SEQ ID NO:1. As indicated herein *inter alia*, the domain region are designated such as to be inclusive of the group, unless otherwise indicated. For example, amino acids 4-12 refers to nine amino acids: amino acids at positions 4, and 12, as well as the seven intervening amino acids in the sequence. Other examples include antigen binding proteins that inhibit binding of activin A to its receptor. An antigen binding protein need not completely inhibit an activin A-induced activity to find use in the present invention; rather, antigen binding proteins that reduce a particular activity of activin A are contemplated for use as well. (Discussions herein of particular mechanisms of action for activin A-binding antigen binding proteins in treating particular diseases are illustrative only, and the methods presented herein are not bound thereby.)

[0087] In another aspect, the present invention provides antigen binding proteins that comprise a light chain variable region selected from the group consisting of A1-A14 or a heavy chain variable region selected from the group consisting of A1-A14, and fragments, derivatives, muteins, and variants thereof. Such an antigen binding protein can be denoted using the nomenclature "LxHy", wherein "x" corresponds to the number of the light chain variable region and "y" corre-

sponds to the number of the heavy chain variable region as they are labeled in the sequences below. That is to say, for example, that "A1HC" denotes the heavy chain variable region of antibody A1; "A1LC" denotes the light chain variable region of antibody A1, and so forth. More generally speaking, "L2H1" refers to an antigen binding protein with a light chain variable region comprising the amino acid sequence of L2 and a heavy chain variable region comprising the amino acid sequence of H1. For clarity, all ranges denoted by at least two members of a group include all members of the group between and including the end range members. Thus, the group range A1-A14, includes all members between A1 and A14, as well as members A1 and A14 themselves. The group range A4-A6 includes members A4, A5, and A6, etc. **[0088]** Also shown below are the locations of the CDRs, or Complementarity Determining Regions (shaded and underlined) that create part of the antigen-binding site, while the Framework Regions (FRs) are the intervening segments of these variable domain sequences. In both light chain variable regions and heavy chain variable regions there are three CDRs (CDR 1-3) and four FRs (FR 1-4). The CDR regions of each light and heavy chain also are grouped by antibody type (A1, A2, A3, etc.). Antigen binding proteins of the invention include, for example, antigen binding proteins having a combination of light chain and heavy chain variable domains selected from the group of combinations consisting of L1H1 (antibody A1), L2H2 (antibody A2), L3H3 (antibody A3), L4H4 (antibody A4), L5H5 (antibody A5), L6H6 (antibody A6), L7H7 (antibody A7), L8H8 (antibody A8), L9H9 (antibody A9), L10H10 (antibody A10), L11H11 (antibody A11), L12H12 (antibody A12), L13H13 (antibody A13), and L14H14 (antibody A14).

Antibodies A1-A14 heavy and light chain variable region polynucleotides (also referred to herein as H1-H14 and L1-L14).

A1 HC

CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG
AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGTTATGGTCTCAGCTGGG
TGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCATCCCTTACA
ATGGTAACACAACTCTGCACAGAACTCCAGGGCAGAGTCACCATGACCACAG
ACACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACA
CGGCCGTGTATTTCTGTGCGAGAGACAGGGGACTACGGTGTCAATTATGATGCTTT
TGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCA (SEQ ID NO:2)

A1 LC

TCCTATGAGGTGACTCAGGCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCA
GCATCACCTGCTCTGGAGATAAATTGGGGGATAAATATGCTTGTTGGTATCAGCA
GAAGCCAGGCCAGTCCCCTGTGCTGGTCATCTATCAAGATAGCAAGCGGCCCTCA
GGGATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGAAACACAGCCACTCTGACCA
TCAGCGGGACCCAGGCTATGGATGAGGCTGACTATTACTGTCAAGGCGTGGGACA
GCAGCACTGCGGTATTTCGGCGGAGGGACCAAGCTGACCGTCCTA (SEQ ID NO:1)

A2 HC

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTG
AGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGTTACGGCATGCACTGGG
TCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATG
GAAGTAATAAATACCATGCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAG
ACAATTCCAAGAACACGCTGTATCTGCAAGTGAACAGCCTGAGAGCCGAGGACA
CGGCTGTGTATTACTGTGTGAGAAGTCCGGAACCTGGAACCTACGACAACCTACTACTA
CGGTCTGGACGCTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAG (SEQ ID
NO:18)

A2 LC

5 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
 TCACCATCACTTGCCCGGGCAAGTCAGGGCATTAGAAATAATTTAGGCTGGTATCA
 GCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATTTATGCTGCATCCAGTTTGCA
AAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAATTCCTCT
 CACAATCAGCAGTCTGCAGCCTGAAGATTTTACAACCTTATTACTGTCTACAGCAT
 10 AATAGTTACCCGTGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA (SEQ ID
 NO:17)

A3 HC

15 GAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTG
 AGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGTAGTTATTGGATGAGCTGGG
 TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGCGTGGCCAACATAAAGCAAGATG
 20 GAAGTGAGGAATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAG
 ACAACGCCAAGAATTCCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
 CGGCTGTGTATTACTGTGCGAGAGGTAGCAGCAGCTGGTACTACTACAACCTACGG
TATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO:34)

A3 LC

25 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
 TCACCATCACTTGCCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCA
 30 GCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCA
AAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAATTCCTCT
 CACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACCTTATTACTGTGCGACAGCAA
AATACTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA (SEQ ID
 35 NO:33)

A4 HC

40 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG
 AAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATCCACTGGG
 TGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCTAACA
GTGGTGGCACAACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGG
 45 ACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACGACA
 CGGCCGTGTATTTCTGTGCGAGAGATTCGGGGGTATAGCAGCAGCTGGCACTTTGA
CTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO:50)

A4 LC

50 GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAGAGCCGG
 CCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTACTGGATACAATA
 55 TTTGGATTGGTACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTG
GGTCTTTTCGGGGCCTCCGGGGTCCCTGACAGGTTCAAGTGGCAGTGGGGTCAGGCA
 CAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTA

CTGCATGCAAGCTCTCCAAACTCCGTGCAGTTTTGGCCAGGGGACCAAGCTGGAG
ATCAAG (SEQ ID NO:49)

5

A5 HC

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15

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTG
TCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAATAGTTTCTACTGGAGCTGGAT
CCGGCAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCTATTACAGTGG
GAGCACCAACTACAATCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAC
GTCCAAGACCCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCC
GTGTATTACTGTGCGAGAGACAGTATAGCAGCCCCCTTTGACTACTGGGGCCAGG
GAACCCTGGTCACCGTCTCCTCAGCTTCCACCAAGGGCCCATCCGTCTTCCCCCT
GGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGT
CAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAATGCGCCCT (SEQ
ID NO:66)

20

A5 LC

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30

GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGG
CCACCATCACCTGCAAGTCCAGCCAGAGTATTTTATACAGTTCCAACAATAAGAA
GTATCTAGTTTGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGATCATTAC
TGGACATCTATGCGGGAATCCGGGGTCCCTGACCGATTCAAGTGGCAGCGGGTCTG
GGACAGATTTCACTCTCACCATCAACAGCCTGCAGGCTGAAGATGTGGCAGTTTA
TTACTGTCAGCAATATTATAGTACTCCGTGGACGTTTCGGCCAAGGGACCAAGGTG
GAAATCAAA (SEQ ID NO:65)

A6 HC

35

40

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTG
TCCCTCACCTGCGCTGTCTATGGTGGGTCCCTTCAGTGCTTACTACTGGAGCTGGAT
CCGCCAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGGAAATCAATCATAGTGG
AGGCACCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAC
GTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCT
GTGTATTACTGTGCGAGAGTACAGTGGCTCGAACTGGCCTACTTTGACTACTGGG
GCCAGGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO:82)

45

A6 LC

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GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
TCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAACTATTTAAATTGGTATCA
GCAGAGACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTACATCCAGTTTGCAA
AGTGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCTGGGACAGATTTCACTCTCA
CCATCAGCAGTCTGCAACCTGAAGATTTTGTAAGTTACTACTGTCAACAGAGTTA
CAGTATTTGCCCCACTTTTCGGCGGCGGGACCAAGGTGGAGAACAAA (SEQ ID
NO:81)

A7 HC

5 CAGGTGCAGCTGGTGGACTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTG
 AGACTCTCCTGTGCAGCGTCTGGATTACCTTCATTAGCTATGGCATGCACTGGG
 TCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATCTGGTATGATG
 10 GAAGTACTGAATACTATGCAGACTCCCGTGAAGGGCCGATTACCATCTCCAGAG
 ACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
 CGGCTGTGTATTACTGTGCGAGAGAGAGGGCAGTGGCTCTACCACTACGGTATGG
 ACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO:98)

A7 LC

15 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
 TCACCATCACTTGCCGGGCAGGTCAGGGCATTAGAAATGATTTAGTCTGGTATCA
 GCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCA
 20 AAGTGGGGTCCCATCAAGGTTACGCGGCAGTGGATCTGGGACAGAATTCCTCT
 CACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTACAACAT
 AATACTTACCCATTCACTTTTCGGCCCTGGGACCAAAGTGGATATCAAA (SEQ ID
 NO:97)

A8 HC

25 CAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCCTCGGAGACCCTG
 TCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAATAGTTTCTACTGGAGCTGGAT
 30 CCGGCAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCTATTACAGTGG
 GAGCACCAACTACAATCCCTCCCTCAAGAGGCGAGTCACCATATCAGTAGACAC
 GTCCAAGACCCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCC
 GTGTATTACTGTGCGAGAGACAGTATAGCAGCCCCCTTTGACTACTGGGGGCCAGG
 35 GAACCCTGGTCACCGTCTCCTCA (SEQ ID NO:114)

A8 LC

40 GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGGCAGAGGG
 CCACCATCACCTGCAAGTCCAGCCAGAGTATTTTATACAGCTCCAACAATAAGAA
 GTATCTAGTTTGGTACCAGCAGAAACCAGGACAGCCTCCTAAGTTGATCATTAC
 TGGACATCTATGCGGGAATCCGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTG
 45 GGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTA
 TTAAGTGTGAGCAATATTATAGTACTCCGTGGACGTTTCGGCCAAGGGACCAAGGTG
 GAAATCAAA (SEQ ID NO:113)

A9 HC

5 CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTG
 AGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGTTACGGCATGCACTGGG
 TCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATG
 GAAGTAATAAATACCATGCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAG
 10 ACAATTCCAAGAACACGCTGTATCTGCAAGTGAACAGCCTGAGAGCCGAGGACA
 CGGCTGTGTATTACTGTGTGAGAAGTCGGAACTGGAACTACGACAACCTACTACTA
 CGGTCTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA (SEQ ID
 NO:130)

A9 LC

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
 TCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATAATTTAGGCTGGTATCA
 20 GCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATTTATGCTGCATCCAGTTTGCA
 AAGTGGGGTCCCATCAAGGTCAGCGGCAGTGGATCTGGGACAGAATTCCTCT
 CACAATCAGCAGCCTGCAGCCTGAAGATTTACAACCTATTACTGTCTACAGCAT
 AATAGTTACCCGTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAA (SEQ ID
 25 NO:129)

A10 HC

30 GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTG
 AAGATCTCCTGTCAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGG
 TGCGCCAGATGCCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTG
 ACTCTGATACCAGATACAGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGA
 35 CAAGTCCATCAGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACAC
 CGCCATGTATT
 ACTGTGCGAGACAAGGACTGGGGTTTGACTACTGGGGCCAGGGAACCCTGGTCA
 CCGTCTCCTCA (SEQ ID NO:146)

A10 LC

45 TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCA
 GCATCACCTGCTCTGGAGAAAAATGGGGAGAGAAAAATATGCTTGTTGGTATCAGC
 AGAAGCCAGGCCAGTCCCCTGTGCTGGTCATCTATCAAGATACCAAGCGGCCCTC
 CGGGATCCCTGAGCGATTCTCTGGCTCCATTCTGGGAACACAGCCACTCTGACC
 ATCAGCGGGACCCAGGCTATGGATGAGGCTGACTATTATTGTAGGCGTGGGAC
 50 AGGAGCACTGTATTTCGGCGGAGGGACCAAGCTGACCGTCCTA (SEQ ID NO:145)

A11 HC

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTG
 TCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGA
 GCTGGATCCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTCTT
 ACAGTGGGAGCACCTACTACAACCCGTCCTCAAGAGTCGAGTTACCATATCAGT
 TGACACGTCTAAGAACCAGTTCTCCCTGAAGCTGAACTCTGTGACTGCCGCGGAC
 ACGGCCGTGTATTACTGTGCGCGCGCTTACGGTGACTATCGCGGCTGGTTCGACC
 CCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO:162)

A11 LC

TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCA
 GCATCACCTGCTCTGGAGATAAATIGGGGGATAAATTGCTTTCTGGTATCAGCT
 GAAGCCAGGCCAGTCCCCTGTGCTGGTCATCTATCAAGATAACAAGCGGCCCTCA
 GGGATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACAGCCACTCTGACCA
 TCAGCGGGACCCAGGCTATGGATGCGGCTGACTTTTACTGTAGGGCGTGGGACA
 GCAGCACTGTGGTATTTCGGCGGAGGGACCAAGCTGACCGTCCTA (SEQ ID
 NO:161)

A12 HL

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTG
 AGACTCTCCTGTGTAGCGTCTGGATTACCTTCAGTGCCTATGGCATGCACTGGG
 TCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATG
 GAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTCATCATCTCCAGAG
 ACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
 CGGCTGTGTATTACTGTGCGAGAAGTCGGAAGTGGAACTACGACTCCTACCAATA
 CGGTTTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA (SEQ ID
 NO:178)

A12 LC

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
 TCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCA
 GCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCA
 AAGTGGGGTCCCATCAAGGTTACGCGGCAGTGGATCTGGGACAGAATTCCTCT
 CACAATCAGCAGCCTGCAGCCTGAAGATTGTGCAACTTATTATTGTCTACAGCAT
 AATAGTTATACGTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAA (SEQ ID
 NO:177)

A13 HC

CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG
 AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGG
 TCGACAGGCCCTGGACAAGGGCTTGAGAGGATGGGATGGATCAGCGCTTACA
 ATGGTAACACAACTATGCACAGAAGTTCCAGGGCAGAGTCACCATGACCACAG
 ACACATCAACGACCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACA
 CGGCCGTGTATTACTGTGCGAGAGATCAAGATTACTATGATAGTAGTGGTTGGGG
 CCACTGGGGCCAGGGAACCCTGGTTCACCGTCTCCTCA (SEQ ID NO:194)

A13 LC

TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCA
 GCATCACCTGCTCTGGAGATAAATTGGGGGATAAATATGTTTGTGTTGATCAGCA
 GAAGCCAGGCCAGTCCCCTGAACTGGTCATCTATCTAGATAACAAGCGGCCCTCA
 GGGATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACAGCCACTCTGACCA
 TCAGCGGGACCCAGGCTATGGATGAGGCTGACTATTACTGTGAGGCGTGGGACA
 GCAGCACGGTATTTCGGCGGAGGGACCAAACTGACCGTCCTG (SEQ ID NO:193)

A14 HC

CAGGTTTCAGCTGGTGCATCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG
 AAGGTCTCCTGCAAGACTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGG
 TCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCCCTTACA
 ATGGTAACACAACTATGCACAGAAGTTCCAGGGCAGAGTCACCATGACCACAG
 ACAAATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGCGATCTGACGACA
 CGGCCGTGTATTACTGTGCGAGAGATCAAGATTACTATGATAGTAGTGGTTGGGA
 CCCCTGGGGCCAGGGAACCCTGGTTCACCGTCTCCTCG (SEQ ID NO:210)

A14 LC

TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCT
 CCATCACCTGCTCTGGAGATAAATTGGGGGATAAATATGCTTCTGTTGATCAGCA
 GAAGCCAGGCCAGTCCCCTGTGCTGGTCTTCTATCATGATACCAAGCGGCCCTCA
 GGGATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACAGCCACTCTGACCA
 TCAGCGGGACCCAGGCTATGGATGAGGCTGACTATCACTGTGAGGCGTGGGACA
 GCAGCACGGTCTTCGGCGGAGGGACCAAGCTGACCGTCCTAC (SEQ ID NO:209)

Antibodies A1-A14 amino acid sequences, light chain variable regions. CDR regions are shaded and underlined;
 the intervening segments or regions are referred to as framework (FR) herein.

A1

SYEVTQAPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIP
 ERFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTAVFGGGTKLTVL (SEQ ID
 NO:9)

A2

DIQMTQSPSSLSASVGDRVTTITCRASQGIRNNLGWYQQKPGKAPKRLIYAASSLQSG
 VPSRFSGSGSGTEFTLTISSLQPEDFTTYCYCLQHNSYPWTFGQGTKVEIK (SEQ ID
 NO:25)

A3

DIQMTQSPSSLSASVGDRVTTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG
 VPSRFSGSGSGTEFTLTISSLQPEDFATYYCRQQNTYPLTFGGGTKVEIK (SEQ ID
 NO:41)

A4

DIVMTQSPSLPVTTPGEPASISCRSSQSLLHSTGYNLYLDWYLQKPGQSPQLLIYLGSRF
 ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPCSFGQGTKLEIK (SEQ
 ID NO:57)

A5

DIVMTQSPDSLAVSLGERATITCKSSQSILYSSNNKKYLVWYQQKPGQPPKLIYWTS
 MRESGVPDRFSGSGSGTDFTLTINSLQAEDVAVYYCQQYYSTPWFPGQGTKVEIK
 (SEQ ID NO:73)

A6

DIQMTQSPSSLSASVGDRVTTITCRASQISNYLNWYQQRPGKAPKLLIYATSSLQSGV
 PSRFSGSGSGTDFTLTISSLQPEDFVSYYCQQSYSISPTFGGGTKVENK (SEQ ID
 NO:89)

A7

DIQMTQSPSSLSASVGDRVTTITCRAGQGIRNDLVWYQQKPGKAPKRLIYAASSLQSG
 VPSRFSGSGSGTEFTLTISSLQPEDFATYYCYLQHNTYPETFGPGTKVDIK (SEQ ID
 NO:105)

A8

DIVMTQSPDSLAVSLGERATITCKSSQSILYSSNNKKYLVWYQQKPGQPPKLIYWTS
 MRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPWFPGQGTKVEIK
 (SEQ ID NO:121)

A9

DIQMTQSPSSLSASVGDRVTTITCRASQGIRNNLGWYQQKPGKAPKRLIYAASSLQSG
 VPSRFSGSGSGTEFTLTISSLQPEDFTTYCYCLQHNSYPWTFGQGTKVEIK (SEQ ID
 NO:137)

A10

SYELTQPPSVSVSPGQTASITCSGEKWGEKYACWYQQKPGQSPVLVIYQDTKRPSGIP
 5 ERFGSGISGNTATLTISGTQAMDEADYYCQAWDRSTVFGGGTKLTVL (SEQ ID
 NO:153)

A11

10 SYELTQPPSVSVSPGQTASITCSGDKLGDKFAFWYQLKPGQSPVLVIYQDNKRPSGIP
 ERFGSGNSGNTATLTISGTQAMDAADFYCQAWDSSTVVFGGGTKLTVL (SEQ ID
 NO:169)

A12

15 DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG
 VPSRFSGSGSGTEFTLTISLQPEDCATYYCLQHNSYTWTFGQGTKVEIK (SEQ ID
 20 NO:185)

A13

25 SYELTQPPSVSVSPGQTASITCSGDKLGDKYVCWYQQKPGQSPELVIYLDNKRPSGIP
 ERFGSGNSGNTATLTISGTQAMDEADYYCQAWDSSTVFGGGTKLTVL (SEQ ID
 NO:201)

A14

30 SYELTQPPSVSVSPGQTASITCSGDKLGDKYAFWYQQKPGQSPVLVFYHDTKRPSGIP
 ERFGSGNSGNTATLTISGTQAMDEADYHCQAWDSSTVFGGGTKLTVL (SEQ ID
 NO:217)

35 Antibodies A1-A14, amino acid sequences of heavy chain variable regions. CDR regions are shaded and underlined,
 the other regions are referred to as framework (FR) herein.

A1

40 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGLSWVRQAPGQGLEWMGWIIPYN
GNTNSAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYFCARDRDYGVNYDAFDI
 WGQGTMTVTVSS (SEQ ID NO:10)

A2

45 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDG
SNKYHADSVKGRFTISRDNSKNTLYLQVNSLRAEDTAVYYCVRSRNWNYDNYYYG
 50 LDVWGQGTTVTVSS (SEQ ID NO:26)

A3

55 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLECVANIKODGS
EEYYVDSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARGSSSWYYNYGMD
VWGQGTTVTVSS (SEQ ID NO:42)

A4

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYIHWVRQAPGQGLEWMGWINPNS
 5 GGTNYAQKFQGRVTMTTRDTSISTAYMELSRLRSDDTAVYFCARDSGYSSSWHFDY
 WGQGTTLVTVSS (SEQ ID NO:58)

A5

10 QVQLQESGPGLVKPSETLSLTCTVSGGGSINSEFYWSWIRQPPGKGLEWIGYIYYSGSTN
YNPSLKSRVTISVDTSKTQFSLKLSSVTAADTAVYYCARDSIAAPFDYWGQGTTLVTV
 SS (SEQ ID NO:74)

A6

15 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSAYYWSWIRQPPGKGLEWIGEINHSGG
TNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARVQWLELAYFDYWGQG
 20 TLVTVSS (SEQ ID NO:90)

A7

25 QVQLVDSGGGVVQPGRSLRLSCAASGFTFISYGMHWVRQAPGKGLEWVAVIWYDG
STEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARERQWLYHYGMDV
 WGQGTTLVTVSS (SEQ ID NO:106)

A8

30 QVQLQESGPGLVKPSETLSLTCTVSGGGSINSEFYWSWIRQPPGKGLEWIGYIYYSGSTN
YNPSLKRRVTISVDTSKTQFSLKLSSVTAADTAVYYCARDSIAAPFDYWGQGTTLVTV
 SS (SEQ ID NO:122)

A9

35 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDG
SNKYHADSVKGRFTISRDN SKNTLYLQVNSLRAEDTAVYYCVRSRNWNVDNYYYG
 40 LDVWGQGTTLVTVSS (SEQ ID NO:138)

A10

45 EVQLVQSGAEVKKPGESLKISCQGSGYSFISYWIGWVRQMPGKGLEWMGIYPGDS
DTRYSPSFQQVTISADKSISTAYLQWSSLKASDTAMYICARQGLGFDYWGQGTTLV
 TVSS (SEQ ID NO:154)

A11

50 QVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWSWIRQHPPGKGLEWIGYISYSGS
TYYNPSLKSRVTISVDTSKNQFSLKLNSVTAADTAVYYCARAYGDYRGWFDPWQ
 55 GTLVTVSS (SEQ ID NO:170)

A12

QVQLVESGGGVVQPGRSLRLSCVASSGFTFSAYGMHWVRQAPGKGLEWVAVIWYD
GSNKYYADSVKGRFIISRDNSKNTLYLQMNSLRAEDTAVYYCARSRNWNYSYQY
GLDVWGQGTITVTVSS (SEQ ID NO:186)

A13

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLERMGWISAYN
GNTNYAQKFQGRVTMTTDTSTTTAYMELRSLRSDDTAVYYCARDQDYYDSSGWG
HWGQGTITVTVSS (SEQ ID NO:202)

A14

QVQLVQSGAEVKKPGASVKVSCKTSGYTFTSYGISWVRQAPGQGLEWMGWISPYN
GNTNYAQKFQGRVTMTTDKSTSTAYMELRSLRSDDTAVYYCARDQDYYDSSGWD
WGQGTITVTVSS (SEQ ID NO:218)

CDR consensus sequences for Antibodies A1-A14.

[0089]

Light Chain	CDR1 Sequence
L4	R S S Q S L L H S T G Y N - Y L D (SEQ ID NO:59)
L5, L8	K S S Q S I L Y S S N N K K Y L V (SEQ ID NO:75)
CONSENSUS:	X ₁ S S Q S X ₂ L X ₃ S X ₄ X ₅ X ₆ X ₇ X ₈ Y L X ₉ (SEQ ID NO:115)

X₁ is an arginine residue or a lysine residue,

X₂ is a leucine residue or a isoleucine residue,

X₃ is a histidine residue or a tyrosine residue,

X₄ is a threonine residue or a serine residue,

X₅ is a glycine residue or an asparagine residue,

X₆ is a tyrosine residue or an asparagine residue,

X₇ is an asparagine residue or a lysine residue,

X₈ is a lysine residue or no residue,

X₉ is an aspartate residue or a valine residue

L2, L9	R A S Q G I R N N L G (SEQ ID NO:27)
L3, L12	R A S Q G I R N D L G (SEQ ID NO:43)
L6	R A S Q S I S N Y L N (SEQ ID NO:91)
L7	R A G Q G I R N D L V (SEQ ID NO:107)
CONSENSUS:	R A X ₁₀ Q X ₁₁ I X ₁₂ N X ₁₃ L X ₁₄ (SEQ ID NO:116)

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X₁₀ is a serine residue or a glycine residue,

X₁₁ is a serine residue or a glycine residue,

5 X₁₂ is a serine residue or an arginine residue,

X₁₃ is a tyrosine residue, an aspartate residue, or an asparagine residue

10 X₁₄ is an aspartate residue, a valine residue, or a glycine residue

L1	S G D K L G D K Y A C (SEQ ID NO:11)
L10	S G E K W G E K Y A C (SEQ ID NO:155)
L11	S G D K L G D K F A F (SEQ ID NO:171)
15 L13	S G D K L G D K Y V C (SEQ ID NO:203)
L14	S G D K L G D K Y A F (SEQ ID NO:219)
CONSENSUS:	S G X ₁₅ K X ₁₆ G X ₁₇ K X ₁₈ X ₁₉ X ₂₀ (SEQ ID NO:123)

20 X₁₅ is a glutamate residue or an aspartate residue,

X₁₆ is a tryptophan residue or a leucine residue,

25 X₁₇ is a glutamate residue or an aspartate residue,

X₁₈ is a tyrosine residue or a phenylalanine residue,

X₁₉ is an alanine residue or a valine residue,

30 X₂₀ is a cysteine residue or a phenylalanine residue

<u>Light Chain</u>	<u>CDR2 Sequence</u>
L2	A T S S L Q S (SEQ ID NO:28)
35 L3, L6, L7, L9, L12	A A S S L Q S (SEQ ID NO:44)
L5, L8	W T S M R E S (SEQ ID NO:76)
L4	L G S F R A S (SEQ ID NO:60)
CONSENSUS:	X ₄₀ X ₄₁ S X ₄₂ X ₄₃ X ₄₄ S (SEQ ID NO:124)

40 X₄₀ is an alanine residue, a tryptophan residue, or a leucine residue,

X₄₁ is a threonine residue, an alanine residue, or a glycine residue,

45 X₄₂ is a serine residue, a methionine residue, or a phenylalanine residue,

X₄₃ is a leucine residue or an arginine residue,

50 X₄₄ is a glutamine residue, a glutamate residue, or an alanine residue

L10	Q D T K R P S (SEQ ID NO:156)
L11	Q D N K R P S (SEQ ID NO:172)
55 L1	Q D S K R P S (SEQ ID NO:12)
L13	L D N K R P S (SEQ ID NO:204)
L14	H D T K R P S (SEQ ID NO:220)

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(continued)

CONSENSUS: X₄₅D X₄₆K R P S (SEQ ID NO:128)

X₄₅ is a glutamine residue, a leucine residue, or a histidine residue,

X₄₆ is a threonine residue, an asparagine residue, or a serine residue

<u>Light Chain</u>	<u>CDR3 Sequence</u>
L1	Q A W D S S T A V (SEQ ID NO:13)
L10	Q A W D R S T - V (SEQ ID NO:157)
L11	Q A W D S S T V V (SEQ ID NO:173)
L13, L14	Q A W D S S T V - (SEQ ID NO:205)

L2	L Q H N S Y P W T (SEQ ID NO:29)
L7	L Q H N T Y P F T (SEQ ID NO:109)
L9	L Q H N S Y P W T (SEQ ID NO:141)
L12	L Q H N S Y T W T (SEQ ID NO:189)

CONSENSUS: L Q H N X₈₁ Y X₈₂ X₈₃ T (SEQ ID NO:131)

X₈₁ is a threonine residue or a serine residue,

X₈₂ is a proline residue or a threonine residue,

X₈₃ is a phenylalanine residue or a tryptophan residue

L3	R Q Q N T Y P L T (SEQ ID NO:45)
L4	M Q A L Q T P C S (SEQ ID NO:61)
L5	Q Q Y Y S T P W T (SEQ ID NO:77)
L6	Q Q S Y S I S P T (SEQ ID NO:93)
L8	Q Q Y Y S T P W T (SEQ ID NO:125)

CONSENSUS: X₇₃Q X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀ (SEQ ID NO:132)

X₇₃ is a methionine residue, a glutamine residue, or an arginine residue,

X₇₄ is an alanine residue, a tyrosine residue, a glutamine residue, or a serine residue,

X₇₅ is a leucine residue, a tyrosine residue, or an asparagine residue,

X₇₆ is a glutamine residue, a serine residue, or a threonine residue,

X₇₇ is a threonine residue, a tyrosine residue, or an isoleucine residue,

X₇₈ is a proline residue or a serine residue,

X₇₉ is a cysteine residue, a tryptophan residue, a leucine residue, or a proline residue,

X₈₀ is a serine residue or a threonine residue

<u>Heavy Chain</u>	<u>CDR1 Sequence</u>
H5	GG S I N S - - F Y W S (SEQ ID NO:78)

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(continued)

	<u>Heavy Chain</u>	<u>CDR1 Sequence</u>
	H6	G G S F S A - - Y Y W S (SEQ ID NO:94)
5	H8	G G S INS - - F Y W S (SEQ ID NO:126)
	H11	G G S I S S G G Y Y W S (SEQ ID NO:174)
	CONSENSUS:	G G S X ₂₁ X ₂₂ X ₂₃ X ₂₄ X ₂₅ X ₂₆ Y W S (SEQ ID NO:134)

10 X₂₁ is an isoleucine residue or a phenylalanine residue

X₂₂ is an asparagine residue or a serine residue

X₂₃ is a serine residue or an alanine residue

15 X₂₄ is a glycine residue or no residue

X₂₅ is a glycine residue or no residue

20 X₂₆ is a phenylalanine residue or a tyrosine residue

	H7	G F T F I S Y G M H (SEQ ID NO:110)
	H4	G Y T F T G Y Y I H (SEQ ID NO:62)
25	H2, H9	G F T F S S Y G M H (SEQ ID NO:30)
	H10	G Y S F T S Y W I G (SEQ ID NO:158)
	CONSENSUS:	G X ₂₇ X ₂₈ F X ₂₉ X ₃₀ Y X ₃₁ X ₃₂ X ₃₃ (SEQ ID NO:139)

30 X₂₇ is a tyrosine residue or a phenylalanine residue,

X₂₈ is a threonine residue or a serine residue,

X₂₉ is a threonine residue, a serine residue, or an isoleucine residue,

35 X₃₀ is a glycine residue or a serine residue,

X₃₁ is a tyrosine residue, a glycine residue, or a tryptophan residue,

40 X₃₂ is an isoleucine residue or a methionine residue,

X₃₃ is a histidine residue or a glycine residue

45	H13	G Y T F T S Y G L S (SEQ ID NO:206)
	H12	G F T F S A Y G M H (SEQ ID NO:190)
	H3	G F T F S S Y W M S (SEQ ID NO:46)
	<u>H1, H14</u>	<u>G Y T F T S Y G I S</u> (SEQ ID NO:14)
50	CONSENSUS:	G X ₃₄ T F X ₃₅ X ₃₆ Y X ₃₇ X ₃₈ X ₃₉ (SEQ ID NO:140)

X₃₄ is a tyrosine residue or a phenylalanine residue,

X₃₅ is a threonine residue or a serine residue,

55 X₃₆ is a serine residue or an alanine residue,

X₃₇ is a glycine residue or a tryptophan residue,

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X₃₈ is a leucine residue, a methionine residue, or an isoleucine residue,

X₃₉ is a serine residue or a histidine residue

5

<u>Heavy Chain</u>	<u>CDR2 Sequence</u>
H11	Y I S Y S G S T Y Y N P S L K S (SEQ ID NO:175)
H5	Y I Y Y S G S T N Y N P S L K S (SEQ ID NO:79)
H6	E I N H S G G T N Y N P S L K S (SEQ ID NO:95)
H8	Y I Y Y S G S T N Y N P S L K R (SEQ ID NO:127)
CONSENSUS:	X ₄₇ I X ₄₈ X ₄₉ S G X ₅₀ T X ₅₁ Y N P S L K X ₅₂ (SEQ ID NO:142)

10

X₄₇ is a tyrosine residue or a glutamate residue,

15

X₄₈ is a serine residue, a tyrosine residue, or an asparagine residue,

X₄₉ is a tyrosine residue or a histidine residue

20

X₅₀ is a serine residue or a glycine residue,

X₅₁ is a tyrosine residue or an asparagine residue,

X₅₂ is a serine residue or an arginine residue

25

H2, H9	V I W Y D G S N K Y H A D S V K G (SEQ ID NO:31)
H12	V I W Y D G S N K Y Y A D S V K G (SEQ ID NO:191)
H3	N I K Q D G S E E Y Y V D S V K G (SEQ ID NO:47)
H7	V I W Y D G S T E Y Y A D S V K G (SEQ ID NO:111)
CONSENSUS:	X ₅₃ I X ₅₄ X ₅₅ D G S X ₅₆ X ₅₇ Y X ₅₈ X ₅₉ D S V K G (SEQ ID NO:179)

30

X₅₃ is an asparagine residue or a valine residue,

35

X₅₄ is a tryptophan residue or a lysine residue,

X₅₅ is a tyrosine residue or a glutamine residue,

X₅₆ is an asparagine residue, a glutamate residue, or a serine residue,

X₅₇ is a lysine residue or a glutamate residue,

X₅₈ is a histidine residue or a tyrosine residue,

40

X₅₉ is an alanine residue or a valine residue

H4	W I N P N S G G T N Y A Q K F Q G (SEQ ID NO:63)
H1	W I I P Y N G N T N S A Q K L Q G (SEQ ID NO:15)
H13	W I S A Y N G N T N Y A Q K F Q G (SEQ ID NO:207)
H14	W I S P Y N G N T N Y A Q K F Q G (SEQ ID NO:223)
H10	I I Y P G D S D T R Y S P S F Q G (SEQ ID NO:159)
CONSENSUS:	X ₆₀ I X ₆₁ X ₆₂ X ₆₃ X ₆₄ X ₆₅ X ₆₆ T X ₆₇ X ₆₈ X ₆₉ X ₇₀ X ₇₁ X ₇₂ Q G (SEQ ID NO:180)

50

X₆₀ is a tryptophan residue or an isoleucine residue,

X₆₁ is an asparagine residue, an isoleucine residue, a serine residue, or a tyrosine residue,

X₆₂ is a proline residue or an alanine residue,

X₆₃ is an asparagine residue, a tyrosine residue, or a glycine residue,

55

X₆₄ is a serine residue, an asparagine residue, or an aspartate residue,

X₆₅ is a glycine residue or a serine residue,

X₆₆ is a glycine residue, an asparagine residue, or an aspartate residue,

X₆₇ is an asparagine residue or an arginine residue,

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X₆₈ is a tyrosine residue or a serine residue,
X₆₉ is an alanine residue or a serine residue
X₇₀ is a glutamine residue or a proline residue,
X₇₁ is a lysine residue or a serine residue,
X₇₂ is a phenylalanine residue or a leucine residue

<u>Heavy Chain</u>	<u>CDR3 Sequence</u>
H5, H8	-- D S I A A P F D Y (SEQ ID NO:80)
H6	V Q W L E L A Y F D Y (SEQ ID NO:96)
H10	--- Q G L G F D Y (SEQ ID NO:160)
CONSENSUS:	X ₈₇ X ₈₈ X ₈₉ X ₉₀ X ₉₁ X ₉₂ X ₉₃ X ₉₄ FDY (SEQ ID NO:187)

X₈₇ is a valine residue or no residue,
X₈₈ is a glutamine residue or no residue,
X₈₉ is an aspartate residue, a tryptophan residue, or no residue,
X₉₀ is a serine residue, a leucine residue, or no residue,
X₉₁ is an isoleucine residue, a glutamate residue, or a glutamine residue,
X₉₂ is an alanine residue, a leucine residue, or a glycine residue,
X₉₃ is an alanine residue or a leucine residue,
X₉₄ is a proline residue, a tyrosine residue, or a glycine residue

H13	D Q D Y Y D S S G W - G H (SEQ ID NO:208)
H14	D Q D Y Y D S S G W - D P (SEQ ID NO:22)
H11	-- A Y G D Y R G W F D P (SEQ ID NO:176)
CONSENSUS:	X ₉₅ X ₉₆ X ₉₇ YX ₉₈ DX ₉₉ X ₁₀₀ GWX ₁₀₁ X ₁₀₂ X ₁₀₃ (SEQ ID NO:188)

X₉₅ is an aspartate residue or no residue,
X₉₆ is a glutamine residue or no residue,
X₉₇ is an aspartate residue or an alanine residue,
X₉₈ is a tyrosine residue or a glycine residue,
X₉₉ is a serine residue or a tyrosine residue,
X₁₀₀ is a serine residue or an arginine residue,
X₁₀₁ is a phenylalanine residue or no residue,
X₁₀₂ is a glycine residue or an aspartate residue,
X₁₀₃ is a histidine residue or a proline residue

H4	--- D S G Y S S S W H F D Y - (SEQ ID NO:64)
H1	--- D R D Y G V N Y D A F D I (SEQ ID NO:16)

(continued)

H2 - S R N W N Y D N Y Y Y G L D V (SEQ ID NO:32)
H12 - S R N W N Y D S Y Q Y G L D V (SEQ ID NO:192)
5 H9 - S R N W N Y D N Y Y Y G L D V (SEQ ID NO:144)
H3 G S S S W Y Y - Y N G M D V - (SEQ ID NO:48)
H7 - E R Q W L Y - - H Y G M D V (SEQ ID NO:112)
CONSENSUS: X₁₀₄X₁₀₅X₁₀₆X₁₀₇X₁₀₈X₁₀₉YX₁₁₀X₁₁₁X₁₁₂X₁₁₃X₁₁₄X₁₁₅X₁₁₆X₁₁₇X₁₁₈(SEQ ID NO:189)

10 X₁₀₄ is a glycine residue or no residue
X₁₀₅ is a serine residue, a glutamate residue, or no residue
X₁₀₆ is an arginine residue, a serine residue, or no residue,
X₁₀₇ is an aspartate residue, an asparagine residue, a serine residue, or a glutamine residue
15 X₁₀₈ is a serine residue, an arginine residue, or a tryptophan residue,
X₁₀₉ is a glycine residue, an aspartate residue, an asparagine residue, a tyrosine residue, or a leucine residue,
X₁₁₀ is a serine residue, a glycine residue, an aspartate residue, or no residue,
X₁₁₁ is a serine residue, a valine residue, an asparagine residue, or a tyrosine residue,
X₁₁₂ is a serine residue, an asparagine residue, a tyrosine residue, or a histidine residue
20 X₁₁₃ is a tryptophan residue, a tyrosine residue, or a glutamine residue,
X₁₁₄ is a histidine residue, an aspartate residue, a tyrosine residue, or no residue,
X₁₁₅ is a phenylalanine residue, an alanine residue, or a glycine residue,
X₁₁₆ an aspartate residue, a phenylalanine residue, a leucine residue, or a methionine residue
X₁₁₇ a tyrosine residue, or an aspartate residue,
25 X₁₁₈ is an isoleucine residue, a valine residue, or no residue

[0090] In one embodiment, the present invention provides an antigen binding protein comprising a light chain variable domain comprising a sequence of amino acids that differs from the sequence of a light chain variable domain selected from the group consisting of L1 through L14 only at 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residues, wherein each such sequence difference is independently either a deletion, insertion, or substitution of one amino acid residue.
30 In another embodiment, the light-chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% identical to the sequence of a light chain variable domain selected from the group consisting of L1-L14. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% identical to a
35 nucleotide sequence that encodes a light chain variable domain selected from the group consisting of L1-L14 (which includes L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, and L14). In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a light chain variable domain selected from the group consisting of L1-L14. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a light chain variable domain selected from the group consisting of L1-L14. In another
40 embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a complement of a light chain polynucleotide of L1-L14.

[0091] In another embodiment, the present invention provides an antigen binding protein comprising a heavy chain variable domain comprising a sequence of amino acids that differs from the sequence of a heavy chain variable domain selected from the group consisting of H1-H14 only at 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residue(s), wherein each such sequence difference is independently either a deletion, insertion, or substitution of one amino acid residue.
45 In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% identical to the sequence of a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% identical to a
50 nucleotide sequence that encodes a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence
55 of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence

of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a complement of a heavy chain polynucleotide selected from SEQ ID NO:10, 26, 42, 58, 74, 90, 106, 122, 136, 154, 170, 186, 202, and 218.

[0092] Particular embodiments of antigen binding proteins of the present invention comprise one or more amino acid sequences that are identical to the amino acid sequences of one or more of the CDRs and/or FRs referenced herein for example, one or more CDR or FR from one or more of SEQ ID Nos: 9-16, 22, 25-32, 36, 41-48-57-62, 64, 73-80, 89-91, 93-96, 105-107, 109-112, 115, 116, 121-128, 131, 132, 134, 137-142, 144, 153-160, 169-176, 179, 180, 185-192, 201-208, 217-220, and 223. In one embodiment, the antigen binding protein comprises a light chain CDR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain CDR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain CDR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain FR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain FR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain FR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain FR4 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR4 sequence illustrated above.

[0093] In one embodiment, the present invention provides an antigen binding protein that comprises one or more CDR sequences that differ from a CDR sequence shown above by no more than 5, 4, 3, 2, or 1 amino acid residues.

[0094] In another embodiment, at least one of the antigen binding protein's CDR3 sequences is a CDR3 sequence from A1-A14, as shown in Table 1 or Table 2. In another embodiment, the antigen binding protein's light chain CDR3 sequence is a light chain CDR3 sequence from A1-A14 as shown Table 1 and the antigen binding protein's heavy chain CDR3 sequence is a heavy chain sequence from A1-A14 as shown in Table 2. In another embodiment, the antigen binding protein comprises 1, 2, 3, 4, or 5 CDR sequence(s) that each independently differs by 6, 5, 4, 3, 2, 1, or 0 single amino acid additions, substitutions, and/or deletions from a CDR sequence of A1-A14, and the antigen binding protein further comprises 1, 2, 3, 4, or 5 CDR sequence(s) that each independently differs by 6, 5, 4, 3, 2, 1, or 0 single amino acid additions, substitutions, and/or deletions from a CDR sequence.

[0095] The light chain CDR's of antibodies A1-A14 are shown below in Table 1, and the heavy chain CDR's of antibodies A1-A14 are shown below in Table 2.

Table 1

Light Chain			
Antibody	CDR1	CDR2	CDR3
A1	SGDKLGDKYAC (SEQ ID NO:11)	QDSKRPS (SEQ ID NO: 12)	QAWDSSTAV (SEQ ID NO: 13)
A2	RASQGIRNNLG (SEQ ID NO:27)	AASSLQS (SEQ ID NO: 28)	LQHNSYPWT (SEQ ID NO: 29)
A3	RASQGIRNDLG (SEQ ID NO:43)	AASSLQS (SEQ ID NO: 44)	RQQNTYPLT (SEQ ID NO: 45)
A4	RSSQSLLHSTGYNYLD (SEQ ID NO: 59)	LGSFRAS (SEQ ID NO: 60)	MQALQTPCS (SEQ ID NO: 61)
A5	KSSQSILYSSNNKKYLV (SEQ ID NO: 75)	WTSMRES (SEQ ID NO: 76)	QQYYSTPWT (SEQ ID NO: 77)
A6	RASQISNYLN (SEQ ID NO:91)	ATSSLQS (SEQ ID NO: 44)	QQSYSISPT (SEQ ID NO:93)
A7	RAGQGIRNDLV (SEQ ID NO:107)	AASSLQS (SEQ ID NO: 44)	LQHNTYPFT (SEQ ID NO: 109)
A8	KSSQSILYSSNNKKYLV (SEQ ID NO: 175)	WTSMRES (SEQ ID NO: 76)	QQYYSTPWT (SEQ ID NO: 125)

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(continued)

Light Chain			
Antibody	CDR1	CDR2	CDR3
A9	RASQGIRNNLG (SEQ ID NO:27)	AASSLQS (SEQ ID NO: 44)	LQHNSYPWT (SEQ ID NO: 141)
A10	SGEKWGEKYAC (SEQ ID NO:155)	QDTKRPS (SEQ ID NO: 156)	QAWDRSTV (SEQ ID NO: 157)
A11	SGDKLGDKFAF (SEQ ID NO:171)	QDNKRPS (SEQ ID NO: 172)	QAWDSSTVV (SEQ ID NO: 173)
A12	RASQGIRNDLG (SEQ ID NO:35)	AASSLQS (SEQ ID NO: 44)	LQHNSYTWT (SEQ ID NO: 189)
A13	SGDKLGDKYVC (SEQ ID NO:203)	LDNKRPS (SEQ ID NO: 204)	QAWDSSTV (SEQ ID NO: 205)
A14	SGDKLGDKYAF (SEQ ID NO:219)	HDTKRPS (SEQ ID NO: 220)	QAWDSSTV (SEQ ID NO: 205)

Table 2

Heavy Chain			
Antibody	CDR1	CDR2	CDR3
A1	GYTFTSYGLS (SEQ ID NO:14)	WIIPYNGNTNSAQKLQ G (SEQ ID NO:15)	DRDYGVNYDAFDI (SEQ ID NO: 16)
A2	GFTFSSYGMH (SEQ ID NO:30)	VIWYDGSNKYHADSV KG (SEQ ID NO:31)	SRNWNVDNYYYGL DV (SEQ ID NO:32)
A3	GFTFSSYWMS (SEQ ID NO:46)	NIKQDGSSEYYVDSVK G (SEQ ID NO:47)	GSSSWYYYNYGMD V (SEQ ID NO:48)
A4	GYTFTGYIIH (SEQ ID NO:62)	WINPNSGGTNYAQKF QG (SEQ ID NO:63)	DSGYSSSWHFDY (SEQ ID NO: 64)
A5	GGINSFYWS (SEQ ID NO:78)	YIYSGSTNYPNPSLKS (SEQ ID NO:79)	DSIAAPFDY (SEQ ID NO:80)
A6	GGSFSAYYWS (SEQ ID NO:94)	EINHSGGTNYPNPSLKS (SEQ ID NO:95)	VQWLELAYFDY (SEQ ID NO:96)
A7	GFTFISYGMH (SEQ ID NO:110)	VIWYDGSSTEYYADSV KG (SEQ ID NO:111)	ERQWLYHYGMDV (SEQ ID NO: 112)
A8	GGINSFYWS (SEQ ID NO:126)	YIYSGSTNYPNPSLKR (SEQ ID NO: 127)	DSIAAPFDY (SEQ ID NO:80)
A9	GFTFSSYGMH (SEQ ID NO:130)	VIWYDGSNKYHADSV KG (SEQ ID NO:31)	SRNWNVDNYYYGL DV (SEQ ID NO:144)
A10	GYSFTSYWIG (SEQ ID NO:158)	IIYPGDS DTRYSPSFQG (SEQ ID NO: 159)	QGLGFDY (SEQ ID NO:160)
A11	GGSISSGGYYWS (SEQ ID NO:174)	YISYSGSTYYPNPSLKS (SEQ ID NO:175)	A YGDYRGWFDP (SEQ ID NO: 176)

(continued)

Heavy Chain			
Antibody	CDR1	CDR2	CDR3
A12	GFTFSAYGMH (SEQ ID NO:190)	VIWYDGSNKYYADSV KG (SEQ ID NO:191)	SRNWNYSYQYGL DV (SEQ ID NO:192)
A13	GYTFTSYGIS (SEQ ID NO:206)	WISAYNGNTNYAQKF QG (SEQ ID NO:207)	DQDYDSSGWDGH (SEQ ID NO: 208)
A14	GYTFTSYGIS (SEQ ID NO:222)	WISPYNGNTNYAQKF QG (SEQ ID NO:223)	DQDYDSSGWDP (SEQ ID NO: 224)

[0096] The nucleotide sequences of A1-A14, or the amino acid sequences of A1-A14, can be altered, for example, by random mutagenesis or by site-directed mutagenesis (e.g., oligonucleotide-directed site-specific mutagenesis) to create an altered polynucleotide comprising one or more particular nucleotide substitutions, deletions, or insertions as compared to the non-mutated polynucleotide. Examples of techniques for making such alterations are described in Walder et al., 1986, Gene 42:133; Bauer et al. 1985, Gene 37:73; Craik, BioTechniques, January 1985, 12-19; Smith et al., 1981, Genetic Engineering: Principles and Methods, Plenum Press; and U.S. Patent Nos. 4,518,584 and 4,737,462. These and other methods can be used to make, for example, derivatives of anti-activin A antibodies that have a desired property, for example, increased affinity, avidity, or specificity for activin A, increased activity or stability *in vivo* or *in vitro*, or reduced *in vivo* side-effects as compared to the underivatized antibody.

[0097] Other derivatives of anti-activin A antibodies within the scope of this invention include covalent or aggregative conjugates of anti-activin A antibodies, or fragments thereof, with other proteins or polypeptides, such as by expression of recombinant fusion proteins comprising heterologous polypeptides fused to the N-terminus or C-terminus of an anti-activin A antibody polypeptide. For example, the conjugated peptide may be a heterologous signal (or leader) polypeptide, e.g., the yeast alpha-factor leader, or a peptide such as an epitope tag. Antigen binding protein-containing fusion proteins can comprise peptides added to facilitate purification or identification of antigen binding protein (e.g., poly-His). An antigen binding protein also can be linked to the FLAG peptide Asp-Tyr-Lys-Asp-Asp-Asp-Lys (DYKDDDDK) (SEQ ID NO:226) as described in Hopp et al., Bio/Technology 6:1204, 1988, and U.S. Patent 5,011,912. The FLAG peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody (mAb), enabling rapid assay and facile purification of expressed recombinant protein. Reagents useful for preparing fusion proteins in which the FLAG peptide is fused to a given polypeptide are commercially available (Sigma, St. Louis, MO).

[0098] Oligomers that contain one or more antigen binding proteins may be employed as activin A antagonists. Oligomers may be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers. Oligomers comprising two or more antigen binding protein are contemplated for use, with one example being a homodimer. Other oligomers include heterodimers, homotrimers, heterotrimers, homotetramers, heterotetramers, etc.

[0099] One embodiment is directed to oligomers comprising multiple antigen binding proteins joined *via* covalent or non-covalent interactions between peptide moieties fused to the antigen binding proteins. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of antigen binding proteins attached thereto, as described in more detail below.

[0100] In particular embodiments, the oligomers comprise from two to four antigen binding proteins. The antigen binding proteins of the oligomer may be in any form, such as any of the forms described above, e.g., variants or fragments. Preferably, the oligomers comprise antigen binding proteins that have activin A binding activity.

[0101] In one embodiment, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al., 1991, PNAS USA 88:10535; Byrn et al., 1990, Nature 344:677; and Hollenbaugh et al., 1992 Curr. Prot.s in Immunol., Suppl. 4, pages 10.19.1 - 10.19.11.

[0102] One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing an activin A binding fragment of an anti-activin A antibody to the Fc region of an antibody. The dimer can be made by, for example, inserting a gene fusion encoding the fusion protein into an appropriate expression vector, expressing the gene fusion in host cells transformed with the recombinant expression vector, and allowing the expressed fusion protein to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield the dimer.

[0103] The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization also are included. Fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

[0104] One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al., 1994, EMBO J. 13:3992-4001. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

[0105] In other embodiments, the variable portion of the heavy and/or light chains of an anti-activin A antibody may be substituted for the variable portion of an antibody heavy and/or light chain.

[0106] Alternatively, the oligomer is a fusion protein comprising multiple antigen binding proteins, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233.

[0107] Another method for preparing oligomeric antigen binding proteins involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., 1988, Science 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al., 1994, FEBS Letters 344:191, hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., 1994, Semin. Immunol. 6:267-78. In one approach, recombinant fusion proteins comprising an anti-activin A antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric anti-activin A antibody fragments or derivatives that form are recovered from the culture supernatant.

[0108] In one aspect, the present invention provides antigen binding proteins that interfere with the binding of activin A to an activin A receptor. Such antigen binding proteins can be made against activin A, or a fragment, variant or derivative thereof, and screened in conventional assays for the ability to interfere with binding of activin A to activin A receptor. Examples of suitable assays are assays that test the antigen binding proteins for the ability to inhibit binding of activin A to cells expressing activin A receptor, or that test antigen binding proteins for the ability to reduce a biological or cellular response that results from the binding of activin A to cell surface activin A receptors. For example, as set forth in Figure 10, as well as the Examples below, antibodies can be screened according to their ability to bind to immobilized antibody surfaces (activin A and/or activin B).

[0109] Antigen-binding fragments of antigen binding proteins of the invention may be produced by conventional techniques. Examples of such fragments include, but are not limited to, Fab and F(ab')₂ fragments. Antibody fragments and derivatives produced by genetic engineering techniques also are contemplated.

[0110] Additional embodiments include chimeric antibodies, e.g., humanized versions of non-human (e.g., murine) monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable domain of a murine antibody (or all or part of the antigen binding site thereof) and a constant domain derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable domain fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al., 1988, Nature 332:323, Liu et al., 1987, Proc. Nat. Acad. Sci. USA 84:3439, Larrick et al., 1989, Bio/Technology 7:934, and Winter et al., 1993, TIPS 14:139. In one embodiment, the chimeric antibody is a CDR grafted antibody. Techniques for humanizing antibodies are discussed in, e.g., U.S. Pat. No.s 5,869,619, 5,225,539, 5,821,337, 5,859,205, 6,881,557, Padlan et al., 1995, FASEB J. 9:133-39, and Tamura et al., 2000, J. Immunol. 164:1432-41.

[0111] Procedures have been developed for generating human or partially human antibodies in non-human animals. For example, mice in which one or more endogenous immunoglobulin genes have been inactivated by various means have been prepared. Human immunoglobulin genes have been introduced into the mice to replace the inactivated mouse genes. Antibodies produced in the animal incorporate human immunoglobulin polypeptide chains encoded by the human genetic material introduced into the animal. In one embodiment, a non-human animal, such as a transgenic mouse, is immunized with an activin A polypeptide, such that antibodies directed against the activin A polypeptide are generated in the animal.

[0112] One example of a suitable immunogen is a soluble human activin A, such as a polypeptide comprising the

extracellular domain of the protein of SEQ ID NO:225, or other immunogenic fragment of the protein of SEQ ID NO:225. Examples of techniques for production and use of transgenic animals for the production of human or partially human antibodies are described in U.S. Patents 5,814,318, 5,569,825, and 5,545,806, Davis et al., 2003, Production of human antibodies from transgenic mice in Lo, ed. Antibody Engineering: Methods and Protocols, Humana Press, NJ:191-200, Kellermann et al., 2002, Curr Opin Biotechnol. 13:593-97, Russel et al., 2000, Infect Immun. 68:1820-26, Gallo et al., 2000, Eur J Immun. 30:534-40, Davis et al., 1999, Cancer Metastasis Rev. 18:421-25, Green, 1999, J Immunol Methods. 231:11-23, Jakobovits, 1998, Advanced Drug Delivery Reviews 31:33-42, Green et al., 1998, J Exp Med. 188:483-95, Jakobovits A, 1998, Exp. Opin. Invest. Drugs. 7:607-14, Tsuda et al., 1997, Genomics. 42:413-21, Mendez et al., 1997, Nat Genet. 15:146-56, Jakobovits, 1994, Curr Biol. 4:761-63, Arbones et al., 1994, Immunity. 1:247-60, Green et al., 1994, Nat Genet. 7:13-21, Jakobovits et al., 1993, Nature. 362:255-58, Jakobovits et al., 1993, Proc Natl Acad Sci USA. 90:2551-55. Chen, J., M. Trounstine, F. W. Alt, F. Young, C. Kurahara, J. Loring, D. Huszar. Inter'l Immunol. 5 (1993): 647-656, Choi et al., 1993, Nature Genetics 4: 117-23, Fishwild et al., 1996, Nature Biotech. 14: 845-51, Harding et al., 1995, Annals of the New York Academy of Sciences, Lonberg et al., 1994, Nature 368: 856-59, Lonberg, 1994, Transgenic Approaches to Human Monoclonal Antibodies in Handbook of Experimental Pharmacology 113: 49-101, Lonberg et al., 1995, Internal Review of Immunology 13: 65-93, Neuberger, 1996, Nature Biotechnology 14: 826, Taylor et al., 1992, Nucleic Acids Res. 20: 6287-95, Taylor et al., 1994, Inter'l Immunol. 6: 579-91, Tomizuka et al., 1997, Nature Genetics 16: 133-43, Tomizuka et al., 2000, Pro. Nat'l Acad. Sci. USA 97: 722-27, Tuaillon et al., 1993, Pro. Nat'l Acad. Sci. USA 90: 3720-24, and Tuaillon et al., 1994, J. Immunol. 152: 2912-20.

[0113] In another aspect, the present invention provides monoclonal antibodies that bind to activin A. Monoclonal antibodies may be produced using any technique known in the art, e.g., by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in the art, e.g., by fusing them with myeloma cells to produce hybridomas. Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6.

[0114] In one embodiment, a hybridoma cell line is produced by immunizing an animal (e.g., a transgenic animal having human immunoglobulin sequences) with an activin A immunogen; harvesting spleen cells from the immunized animal; fusing the harvested spleen cells to a myeloma cell line, thereby generating hybridoma cells; establishing hybridoma cell lines from the hybridoma cells, and identifying a hybridoma cell line that produces an antibody that binds an activin A polypeptide. Such hybridoma cell lines, and anti-activin A monoclonal antibodies produced by them, are encompassed by the present invention.

[0115] Monoclonal antibodies secreted by a hybridoma cell line can be purified using any technique known in the art. Hybridomas or mAbs may be further screened to identify mAbs with particular properties, such as the ability to block an activin A-induced activity. Examples of such screens are provided in the examples below.

[0116] Molecular evolution of the complementarity determining regions (CDRs) in the center of the antibody binding site also has been used to isolate antibodies with increased affinity, for example, antibodies having increased affinity for c-erbB-2, as described by Schier et al., 1996, J. Mol. Biol. 263:551. Accordingly, such techniques are useful in preparing antibodies to activin A.

[0117] Antigen binding proteins directed against an activin A can be used, for example, in assays to detect the presence of activin A polypeptides, either *in vitro* or *in vivo*. The antigen binding proteins also may be employed in purifying activin A proteins by immunoaffinity chromatography. Those antigen binding proteins that additionally can block binding of activin A may be used to inhibit a biological activity that results from such binding. Blocking antigen binding proteins can be used in the methods of the present invention. Such antigen binding proteins that function as activin A antagonists may be employed in treating any activin A-related condition, including but not limited to cachexia. In one embodiment, a human anti-activin A monoclonal antibody generated by procedures involving immunization of transgenic mice is employed in treating such conditions.

[0118] Although human, partially human, or humanized antibodies will be suitable for many applications, particularly those involving administration of the antibody to a human subject, other types of antigen binding proteins will be suitable for certain applications. The non-human antibodies of the invention can be, for example, derived from any antibody-producing animal, such as mouse, rat, rabbit, goat, donkey, or non-human primate (such as monkey (e.g., cynomolgous or rhesus monkey) or ape (e.g., chimpanzee)). Non-human antibodies of the invention can be used, for example, in *in vitro* and cell-culture based applications, or any other application where an immune response to the antibody of the invention does not occur, is insignificant, can be prevented, is not a concern, or is desired. In one embodiment, a non-human antibody of the invention is administered to a non-human subject. In another embodiment, the non-human antibody does not elicit an immune response in the non-human subject. In another embodiment, the non-human antibody

is from the same species as the non-human subject, *e.g.*, a mouse antibody of the invention is administered to a mouse. An antibody from a particular species can be made by, for example, immunizing an animal of that species with the desired immunogen (*e.g.*, a soluble activin A polypeptide) or using an artificial system for generating antibodies of that species (*e.g.*, a bacterial or phage display-based system for generating antibodies of a particular species), or by converting

an antibody from one species into an antibody from another species by replacing, *e.g.*, the constant region of the antibody with a constant region from the other species, or by replacing one or more amino acid residues of the antibody so that it more closely resembles the sequence of an antibody from the other species. In one embodiment, the antibody is a chimeric antibody comprising amino acid sequences derived from antibodies from two or more different species.

[0119] Antigen binding proteins may be prepared by any of a number of conventional techniques. For example, they may be purified from cells that naturally express them (*e.g.*, an antibody can be purified from a hybridoma that produces it), or produced in recombinant expression systems, using any technique known in the art. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

[0120] Any expression system known in the art can be used to make the recombinant polypeptides of the invention. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacilli*. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, *Cell* 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al., 1991, *EMBO J.* 10: 2821. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985).

[0121] The transformed cells can be cultured under conditions that promote expression of the polypeptide, and the polypeptide recovered by conventional protein purification procedures. One such purification procedure includes the use of affinity chromatography, *e.g.*, over a matrix having all or a portion (*e.g.*, the extracellular domain) of activin A bound thereto. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti-activin A antibody polypeptides substantially free of contaminating endogenous materials.

[0122] Antigen binding proteins may be prepared, and screened for desired properties, by any of a number of known techniques. Certain of the techniques involve isolating a nucleic acid encoding a polypeptide chain (or portion thereof) of an antigen binding protein of interest (*e.g.*, an anti-activin A antibody), and manipulating the nucleic acid through recombinant DNA technology. The nucleic acid may be fused to another nucleic acid of interest, or altered (*e.g.*, by mutagenesis or other conventional techniques) to add, delete, or substitute one or more amino acid residues, for example.

[0123] In one aspect, the present invention provides antigen-binding fragments of an anti-activin A antibody of the invention. Such fragments can consist entirely of antibody-derived sequences or can comprise additional sequences. Examples of antigen-binding fragments include Fab, F(ab')₂, single chain antibodies, diabodies, triabodies, tetrabodies, and domain antibodies. Other examples are provided in Lunde et al., 2002, *Biochem. Soc. Trans.* 30:500-06.

[0124] Single chain antibodies may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V_L and V_H). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (*e.g.*, dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, *Prot. Eng.* 10:423; Kortt et al., 2001, *Biomol. Eng.* 18:95-108). By combining different V_L and V_H-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum et al., 2001, *Biomol. Eng.* 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Patent No. 4,946,778; Bird, 1988, *Science* 242:423; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879; Ward et al., 1989, *Nature* 334:544; de Graaf et al., 2002, *Methods Mol Biol.* 178:379-87. Single chain antibodies derived from antibodies provided herein include, but are not limited to, scFvs comprising the variable domain combinations L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14 are encompassed by the present invention.

[0125] Antigen binding proteins (*e.g.*, antibodies, antibody fragments, and antibody derivatives) of the invention can comprise any constant region known in the art. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, *e.g.*, a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant regions, *e.g.*, a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In one embodiment, the light or heavy chain constant region is a fragment, derivative, variant, or mutein of a naturally occurring constant region.

[0126] Techniques are known for deriving an antibody of a different subclass or isotype from an antibody of interest,

i.e., subclass switching. Thus, IgG antibodies may be derived from an IgM antibody, for example, and *vice versa*. Such techniques allow the preparation of new antibodies that possess the antigen-binding properties of a given antibody (the parent antibody), but also exhibit biological properties associated with an antibody isotype or subclass different from that of the parent antibody. Recombinant DNA techniques may be employed. Cloned DNA encoding particular antibody polypeptides may be employed in such procedures, *e.g.*, DNA encoding the constant domain of an antibody of the desired isotype. See also Lantto et al., 2002, *Methods Mol. Biol.* 178:303-16.

[0127] In one embodiment, an antigen binding protein of the invention comprises the IgG1 heavy chain domain of any of A1-A14 (H1-H14) or a fragment of the IgG1 heavy chain domain of any of A1-A14 (H1-H14). In another embodiment, an antigen binding protein of the invention comprises the kappa light chain constant chain region of A1-A14 (L1-L14), or a fragment of the kappa light chain constant region of A1-A14 (L1-L14). In another embodiment, an antigen binding protein of the invention comprises both the IgG1 heavy chain domain, or a fragment thereof, of A1-A14 (L1-L14) and the kappa light chain domain, or a fragment thereof, of A1-A14 (L1-L14).

[0128] Accordingly, the antigen binding proteins of the present invention include those comprising, for example, the variable domain combinations L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14, having a desired isotype (for example, IgA, IgG1, IgG2, IgG3, IgG4, IgM, IgE, and IgD) as well as Fab or F(ab')₂ fragments thereof. Moreover, if an IgG4 is desired, it may also be desired to introduce a point mutation (CPSCP -> CPPCP) in the hinge region as described in Bloom et al., 1997, *Protein Science* 6:407, incorporated by reference herein) to alleviate a tendency to form intra-H chain disulfide bonds that can lead to heterogeneity in the IgG4 antibodies.

[0129] In one embodiment, the antigen binding protein has a K_{off} of $1 \times 10^{-4} \text{ s}^{-1}$ or lower. In another embodiment, the K_{off} is $5 \times 10^{-5} \text{ s}^{-1}$ or lower. In another embodiment, the K_{off} is substantially the same as an antibody having a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14. In another embodiment, the antigen binding protein binds to activin A with substantially the same K_{off} as an antibody that comprises one or more CDRs from an antibody having a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14. In another embodiment, the antigen binding protein binds to activin A with substantially the same K_{off} as an antibody that comprises one of the amino acid sequences illustrated above. In another embodiment, the antigen binding protein binds to activin A with substantially the same K_{off} as an antibody that comprises one or more CDRs from an antibody that comprises one of the amino acid sequences illustrated above.

[0130] As used herein, the term human activin A is intended to include the protein of SEQ ID NO:1 and allelic variants thereof. Activin A can be purified from host cells that have been transfected by a gene encoding activin A by elution of filtered supernatant of host cell culture fluid using a Heparin HP column, using a salt gradient.

[0131] The term "antibody" refers to an intact antibody, or a binding fragment thereof. An antibody may comprise a complete antibody molecule (including polyclonal, monoclonal, chimeric, humanized, or human versions having full length heavy and/or light chains), or comprise an antigen binding fragment thereof. Antibody fragments include F(ab')₂, Fab, Fab', Fv, Fc, and Fd fragments, and can be incorporated into single domain antibodies, single-chain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (See *e.g.*, Hollinger and Hudson, 2005, *Nature Biotech.*, 23, 9, 1126-1136). Antibody polypeptides are also disclosed in U. S. Patent No. 6,703,199, including fibronectin polypeptide monobodies. Other antibody polypeptides are disclosed in U.S. Patent Publication 2005/0238646, which are single-chain polypeptides.

[0132] Antigen binding fragments derived from an antibody can be obtained, for example, by proteolytic hydrolysis of the antibody, for example, pepsin or papain digestion of whole antibodies according to conventional methods. By way of example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment termed F(ab')₂. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Patent No. 4,331,647, Nisonoff et al., *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman et al., in *Methods in Enzymology* 1:422 (Academic Press 1967); and by Andrews, S.M. and Titus, J.A. in *Current Protocols in Immunology* (Coligan J.E., et al., eds), John Wiley & Sons, New York (2003), pages 2.8.1-2.8.10 and 2.10A.1-2.10A.5. Other methods for cleaving antibodies, such as separating heavy chains to form monovalent light-heavy chain fragments (Fd), further cleaving of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

[0133] An antibody fragment may also be any synthetic or genetically engineered protein. For example, antibody fragments include isolated fragments consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (scFv proteins).

[0134] Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs (also termed "minimal recognition units", or "hypervariable region") can be obtained by constructing polynucleotides that encode the CDR of interest. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA of antibody-producing cells as a template (see, for example, Larrick et al., *Methods: A Companion to Methods in Enzymology* 2:106, 1991; Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), page 166 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch et al., (eds.), page 137 (Wiley-Liss, Inc. 1995)).

[0135] Thus, in one embodiment, the binding agent comprises at least one CDR as described herein. The binding agent may comprise at least two, three, four, five or six CDR's as described herein. The binding agent further may comprise at least one variable region domain of an antibody described herein. The variable region domain may be of any size or amino acid composition and will generally comprise at least one CDR sequence responsible for binding to human activin A, for example CDR-H1, CDR-H2, CDR-H3 and/or the light chain CDRs specifically described herein and which is adjacent to or in frame with one or more framework sequences. In general terms, the variable (V) region domain may be any suitable arrangement of immunoglobulin heavy (V_H) and/or light (V_L) chain variable domains. Thus, for example, the V region domain may be monomeric and be a V_H or V_L domain, which is capable of independently binding human activin A with an affinity at least equal to $1 \times 10^{-7}M$ or less as described below. Alternatively, the V region domain may be dimeric and contain V_H - V_H , V_H - V_L , or V_L - V_L dimers. The V region dimer comprises at least one V_H and at least one V_L chain that may be non-covalently associated (hereinafter referred to as F_V). If desired, the chains may be covalently coupled either directly, for example via a disulfide bond between the two variable domains, or through a linker, for example a peptide linker, to form a single chain F_v (scF_v).

[0136] The variable region domain may be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain that has been created using recombinant DNA engineering techniques. Such engineered versions include those created, for example, from a specific antibody variable region by insertions, deletions, or changes in or to the amino acid sequences of the specific antibody. Particular examples include engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from a first antibody and the remainder of the variable region domain from a second antibody.

[0137] The variable region domain may be covalently attached at a C-terminal amino acid to at least one other antibody domain or a fragment thereof. Thus, for example, a V_H domain that is present in the variable region domain may be linked to an immunoglobulin CH1 domain, or a fragment thereof. Similarly a V_L domain may be linked to a C_K domain or a fragment thereof. In this way, for example, the antibody may be a Fab fragment wherein the antigen binding domain contains associated V_H and V_L domains covalently linked at their C-termini to a CH1 and C_K domain, respectively. The CH1 domain may be extended with further amino acids, for example to provide a hinge region or a portion of a hinge region domain as found in a Fab' fragment, or to provide further domains, such as antibody CH2 and CH3 domains.

[0138] As described herein, antibodies comprise at least one of these CDRs. For example, one or more CDR may be incorporated into known antibody framework regions (IgG1, IgG2, etc.), or conjugated to a suitable vehicle to enhance the half-life thereof. Suitable vehicles include, but are not limited to Fc, polyethylene glycol (PEG), albumin, transferrin, and the like. These and other suitable vehicles are known in the art. Such conjugated CDR peptides may be in monomeric, dimeric, tetrameric, or other form. In one embodiment, one or more water-soluble polymer is bonded at one or more specific position, for example at the amino terminus, of a binding agent.

[0139] In certain preferred embodiments, an antibody comprises one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. See, e.g., U.S. Pat. Nos. 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192 and 4,179,337. In certain embodiments, a derivative binding agent comprises one or more of monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, as well as mixtures of such polymers. In certain embodiments, one or more water-soluble polymer is randomly attached to one or more side chains. In certain embodiments, PEG can act to improve the therapeutic capacity for a binding agent, such as an antibody. Certain such methods are discussed, for example, in U.S. Pat. No. 6,133,426, which is hereby incorporated by reference for any purpose.

[0140] It will be appreciated that an antibody of the present invention may have at least one amino acid substitution, providing that the antibody retains binding specificity. Therefore, modifications to the antibody structures are encompassed within the scope of the invention. These may include amino acid substitutions, which may be conservative or non-conservative, that do not destroy the activin A binding capability of an antibody. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties. A conservative amino acid substitution may also involve a substitution of a native amino

acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position.

[0141] Non-conservative substitutions may involve the exchange of a member of one class of amino acids or amino acid mimetics for a member from another class with different physical properties (e.g. size, polarity, hydrophobicity, charge). Such substituted residues may be introduced into regions of the human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule.

[0142] Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change may be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[0143] A skilled artisan will be able to determine suitable variants of the polypeptide as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[0144] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues which are important for activity or structure in similar proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[0145] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules.

[0146] A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4):422-427 (1996), Chou et al., Biochem., 13(2):222-245 (1974); Chou et al., Biochem., 113(2):211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol., 47:45-148 (1978); Chou et al., Ann. Rev. Biochem., 47:251-276 and Chou et al., Biophys. J., 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

[0147] Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-19 (1996)), "profile analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov et al., Meth. Enzym., 183:146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Holm, supra (1999), and Brenner, supra (1997)).

[0148] In certain embodiments, variants of antibodies include glycosylation variants wherein the number and/or type of glycosylation site has been altered compared to the amino acid sequences of a parent polypeptide. In certain embodiments, variants comprise a greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred antibody variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) as compared to the parent amino acid sequence. Cysteine variants may be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions

resulting from unpaired cysteines.

[0149] Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of antibodies to activin A, or to increase or decrease the affinity of the antibodies to activin A described herein.

[0150] According to certain embodiments, preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (4) confer or modify other physiochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In certain embodiments, a conservative amino acid substitution typically may not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991), which are each incorporated herein by reference.

[0151] In certain embodiments, antibodies of the invention may be chemically bonded with polymers, lipids, or other moieties.

[0152] The binding agents may comprise at least one of the CDRs described herein incorporated into a biocompatible framework structure. In one example, the biocompatible framework structure comprises a polypeptide or portion thereof that is sufficient to form a conformationally stable structural support, or framework, or scaffold, which is able to display one or more sequences of amino acids that bind to an antigen (e.g., CDRs, a variable region, etc.) in a localized surface region. Such structures can be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or can have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally occurring polypeptide or fold. These scaffolds can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus.

[0153] Typically the biocompatible framework structures are based on protein scaffolds or skeletons other than immunoglobulin domains. For example, those based on fibronectin, ankyrin, lipocalin, neocarzinostatin, cytochrome b, CP1 zinc finger, PST1, coiled coil, LACI-D1, Z domain and tendamistat domains may be used (See e.g., Nygren and Uhlen, 1997, *Curr. Opin. in Struct. Biol.*, 7, 463-469).

[0154] It will be appreciated that the antibodies of the invention include the humanized antibodies described herein. Humanized antibodies such as those described herein can be produced using techniques known to those skilled in the art (Zhang, W., et al., *Molecular Immunology*. 42(12):1445-1451, 2005; Hwang W. et al., *Methods*. 36(1):35-42, 2005; Dall'Acqua WF, et al., *Methods* 36(1):43-60, 2005; and Clark, M., *Immunology Today*. 21(8):397-402, 2000).

[0155] Additionally, one skilled in the art will recognize that suitable binding agents include portions of these antibodies, such as one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 as specifically disclosed herein. At least one of the regions of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 may have at least one amino acid substitution, provided that the antibody retains the binding specificity of the non-substituted CDR. The non-CDR portion of the antibody may be a non-protein molecule, wherein the binding agent cross-blocks the binding of an antibody disclosed herein to activin A and/or neutralizes activin A. The non-CDR portion of the antibody may be a non-protein molecule in which the antibody exhibits a similar binding pattern to human activin A peptides in a competition binding assay as that exhibited by at least one of antibodies A1-A14, and/or neutralizes activin A. The non-CDR portion of the antibody may be composed of amino acids, wherein the antibody is a recombinant binding protein or a synthetic peptide, and the recombinant binding protein cross-blocks the binding of an antibody disclosed herein to activin A and/or neutralizes activin A. The non-CDR portion of the antibody may be composed of amino acids, wherein the antibody is a recombinant antibody, and the recombinant antibody exhibits a similar binding pattern to human activin A peptides in the human activin A peptide epitope competition binding assay (described hereinbelow) as that exhibited by at least one of the antibodies A1-A14, and/or neutralizes activin A.

[0156] Where an antibody comprises one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 as described above, it may be obtained by expression from a host cell containing DNA coding for these sequences. A DNA coding for each CDR sequence may be determined on the basis of the amino acid sequence of the CDR and synthesized together with any desired antibody variable region framework and constant region DNA sequences using oligonucleotide synthesis techniques, site-directed mutagenesis and polymerase chain reaction (PCR) techniques as appropriate. DNA coding for variable region frameworks and constant regions is widely available to those skilled in the art from genetic sequences databases such as GenBank®.

[0157] Once synthesized, the DNA encoding an antibody of the invention or fragment thereof may be propagated and expressed according to any of a variety of well-known procedures for nucleic acid excision, ligation, transformation, and

transfection using any number of known expression vectors. Thus, in certain embodiments expression of an antibody fragment may be preferred in a prokaryotic host, such as *Escherichia coli* (see, e.g., Pluckthun et al., 1989 Methods Enzymol. 178:497-515). In certain other embodiments, expression of the antibody or a fragment thereof may be preferred in a eukaryotic host cell, including yeast (e.g., *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*), animal cells (including mammalian cells) or plant cells. Examples of suitable animal cells include, but are not limited to, myeloma (such as a mouse NSO line), COS, CHO, or hybridoma cells. Examples of plant cells include tobacco, corn, soybean, and rice cells.

[0158] One or more replicable expression vectors containing DNA encoding an antibody variable and/or constant region may be prepared and used to transform an appropriate cell line, for example, a non-producing myeloma cell line, such as a mouse NSO line or a bacteria, such as *E. coli*, in which production of the antibody will occur. In order to obtain efficient transcription and translation, the DNA sequence in each vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operatively linked to the variable domain sequence. Particular methods for producing antibodies in this way are generally well-known and routinely used. For example, basic molecular biology procedures are described by Maniatis et al. (Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, New York, 1989; see also Maniatis et al., 3rd ed., Cold Spring Harbor Laboratory, New York, (2001)). DNA sequencing can be performed as described in Sanger et al. (PNAS 74:5463, (1977)) and the Amersham International plc sequencing handbook, and site directed mutagenesis can be carried out according to methods known in the art (Kramer et al., Nucleic Acids Res. 12:9441, (1984); Kunkel Proc. Natl. Acad. Sci. USA 82:488-92 (1985); Kunkel et al., Methods in Enzymol. 154:367-82 (1987); the Anglian Biotechnology Ltd. handbook). Additionally, numerous publications describe techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors, and transformation and culture of appropriate cells (Mountain A and Adair, J R in Biotechnology and Genetic Engineering Reviews (ed. Tombs, M P, 10, Chapter 1, 1992, Intercept, Andover, UK); "Current Protocols in Molecular Biology", 1999, F.M. Ausubel (ed.), Wiley Interscience, New York).

[0159] Where it is desired to improve the affinity of antibodies according to the invention containing one or more of the above-mentioned CDRs can be obtained by a number of affinity maturation protocols including maintaining the CDRs (Yang et al., J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks et al., Bio/Technology, 10, 779-783, 1992), use of mutation strains of *E. coli*. (Low et al., J. Mol. Biol., 250, 350-368, 1996), DNA shuffling (Patten et al., Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson et al., J. Mol. Biol., 256, 7-88, 1996) and sexual PCR (Cramer, et al., Nature, 391, 288-291, 1998). All of these methods of affinity maturation are discussed by Vaughan et al. (Nature Biotech., 16, 535-539, 1998).

[0160] Other antibodies according to the invention may be obtained by conventional immunization and cell fusion procedures as described herein and known in the art. Monoclonal antibodies of the invention may be generated using a variety of known techniques. In general, monoclonal antibodies that bind to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler et al., Nature 256:495, 1975; Coligan et al. (eds.), Current Protocols in Immunology, 1:2.5.12.6.7 John Wiley & Sons 1991); U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.) (1980); and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press (1988); Picksley et al., "Production of monoclonal antibodies against proteins expressed in *E. coli*," in DNA Cloning 2: Expression Systems, 2nd Edition, Glover et al. (eds.), page 93 (Oxford University Press 1995)). Antibody fragments may be derived therefrom using any suitable standard technique such as proteolytic digestion, or optionally, by proteolytic digestion (for example, using papain or pepsin) followed by mild reduction of disulfide bonds and alkylation. Alternatively, such fragments may also be generated by recombinant genetic engineering techniques as described herein.

[0161] Monoclonal antibodies can be obtained by injecting an animal, for example, a rat, hamster, a rabbit, or preferably a mouse, including for example a transgenic or a knockout, as known in the art, with an immunogen comprising human activin A of SEQ ID NO:66, or a fragment thereof, according to methods known in the art and described herein. The presence of specific antibody production may be monitored after the initial injection and/or after a booster injection by obtaining a serum sample and detecting the presence of an antibody that binds to human activin A or peptide using any one of several immunodetection methods known in the art and described herein. From animals producing the desired antibodies, lymphoid cells, most commonly cells from the spleen or lymph node, are removed to obtain B-lymphocytes. The B lymphocytes are then fused with a drug-sensitized myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal and that optionally has other desirable properties (e.g., inability to express endogenous Ig gene products, e.g., P3X63 - Ag 8.653 (ATCC No. CRL 1580); NSO, SP20) to produce hybridomas, which are immortal eukaryotic cell lines.

[0162] The lymphoid (e.g., spleen) cells and the myeloma cells may be combined for a few minutes with a membrane fusion-promoting agent, such as polyethylene glycol or a nonionic detergent, and then plated at low density on a selective medium that supports the growth of hybridoma cells but not unfused myeloma cells. A preferred selection media is HAT (hypoxanthine, aminopterin, thymidine). After a sufficient time, usually about one to two weeks, colonies of cells are

observed. Single colonies are isolated, and antibodies produced by the cells may be tested for binding activity to human activin A, using any one of a variety of immunoassays known in the art and described herein. The hybridomas are cloned (e.g., by limited dilution cloning or by soft agar plaque isolation) and positive clones that produce an antibody specific to activin A are selected and cultured. The monoclonal antibodies from the hybridoma cultures may be isolated from the supernatants of hybridoma cultures.

[0163] An alternative method for production of a murine monoclonal antibody is to inject the hybridoma cells into the peritoneal cavity of a syngeneic mouse, for example, a mouse that has been treated (e.g., pristane-primed) to promote formation of ascites fluid containing the monoclonal antibody. Monoclonal antibodies can be isolated and purified by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines et al., "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology*, Vol. 10, pages 79-104 (The Humana Press, Inc. 1992)). Monoclonal antibodies may be purified by affinity chromatography using an appropriate ligand selected based on particular properties of the antibody (e.g., heavy or light chain isotype, binding specificity, etc.). Examples of a suitable ligand, immobilized on a solid support, include Protein A, Protein G, an anticonstant region (light chain or heavy chain) antibody, an anti-idiotypic antibody, and a TGF-beta binding protein, or fragment or variant thereof.

[0164] An antibody of the present invention may also be a fully human monoclonal antibody. An isolated fully human antibody is provided that specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin A, wherein the antigen binding protein possesses at least one *in vivo* biological activity of a human anti-activin A antibody. The biological activity may be attenuation of cachexia, for example cachexia in colon cancer, such as in a mouse model of colon cancer described herein. The cachexia amenable to such treatment is associated with loss of body weight, loss of muscle mass, and/or loss of fat mass. The cachexia may be associated with rheumatoid arthritis, such as in a collagen-induced animal model of rheumatoid arthritis. Treatment with a fully human antibody described herein ameliorates the loss of body weight, the loss of muscle mass, and/or the loss of fat mass *in vivo* in a collagen-induced animal model of rheumatoid arthritis. A fully human antibody described herein ameliorates the loss of body weight in a AAV-activin A transfected animal model. A fully human antibody described herein, that specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin A, inhibits the binding of activin A to activin A receptor *in vitro*. A fully human isolated antibody that specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin A, inhibits the binding of activin A to activin A receptor *in vivo*.

[0165] Fully human monoclonal antibodies may be generated by any number of techniques with which those having ordinary skill in the art will be familiar. Such methods include, but are not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (e.g., containing B lymphocytes), *in vitro* immunization of human B-cells, fusion of spleen cells from immunized transgenic mice carrying inserted human immunoglobulin genes, isolation from human immunoglobulin V region phage libraries, or other procedures as known in the art and based on the disclosure herein. For example, fully human monoclonal antibodies may be obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. Methods for obtaining fully human antibodies from transgenic mice are described, for example, by Green et al., *Nature Genet.* 7:13, 1994; Lonberg et al., *Nature* 368:856, 1994; Taylor et al., *Int. Immun.* 6:579, 1994; U.S. Patent No. 5,877,397; Bruggemann et al., 1997 *Curr. Opin. Biotechnol.* 8:455-58; Jakobovits et al., 1995 *Ann. N. Y. Acad. Sci.* 764:525-35. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci (see also Bruggemann et al., *Curr. Opin. Biotechnol.* 8:455-58 (1997)). For example, human immunoglobulin transgenes may be mini-gene constructs, or transloci on yeast artificial chromosomes, which undergo B-cell-specific DNA rearrangement and hypermutation in the mouse lymphoid tissue. Fully human monoclonal antibodies may be obtained by immunizing the transgenic mice, which may then produce human antibodies specific for activin A. Lymphoid cells of the immunized transgenic mice can be used to produce human antibody-secreting hybridomas according to the methods described herein. Polyclonal sera containing fully human antibodies may also be obtained from the blood of the immunized animals.

[0166] Another method for generating human antibodies of the invention includes immortalizing human peripheral blood cells by EBV transformation. See, e.g., U.S. Patent No. 4,464,456. Such an immortalized B-cell line (or lymphoblastoid cell line) producing a monoclonal antibody that specifically binds to activin A can be identified by immunodetection methods as provided herein, for example, an ELISA, and then isolated by standard cloning techniques. The stability of the lymphoblastoid cell line producing an anti-activin A antibody may be improved by fusing the transformed cell line with a murine myeloma to produce a mouse-human hybrid cell line according to methods known in the art (see, e.g., Glasky et al., *Hybridoma* 8:377-89 (1989)). Still another method to generate human monoclonal antibodies is *in vitro* immunization, which includes priming human splenic B-cells with human activin A, followed by fusion of primed B-cells with a heterohybrid fusion partner. See, e.g., Boerner et al., 1991 *J. Immunol.* 147:86-95.

[0167] In certain embodiments, a B-cell that is producing an anti-human activin A antibody is selected and the light

chain and heavy chain variable regions are cloned from the B-cell according to molecular biology techniques known in the art (WO 92/02551; U.S. Patent 5,627,052; Babcook et al., Proc. Natl. Acad. Sci. USA 93:7843-48 (1996)) and described herein. B-cells from an immunized animal may be isolated from the spleen, lymph node, or peripheral blood sample by selecting a cell that is producing an antibody that specifically binds to activin A. B-cells may also be isolated from humans, for example, from a peripheral blood sample. Methods for detecting single B-cells that are producing an antibody with the desired specificity are well known in the art, for example, by plaque formation, fluorescence-activated cell sorting, *in vitro* stimulation followed by detection of specific antibody, and the like. Methods for selection of specific antibody-producing B-cells include, for example, preparing a single cell suspension of B-cells in soft agar that contains human activin A. Binding of the specific antibody produced by the B-cell to the antigen results in the formation of a complex, which may be visible as an immunoprecipitate. After the B-cells producing the desired antibody are selected, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA according to methods known in the art and described herein.

[0168] An additional method for obtaining antibodies of the invention is by phage display. See, e.g., Winter et al., 1994 Annu. Rev. Immunol. 12:433-55; Burton et al., 1994 Adv. Immunol. 57:191-280. Human or murine immunoglobulin variable region gene combinatorial libraries may be created in phage vectors that can be screened to select Ig fragments (Fab, Fv, sFv, or multimers thereof) that bind specifically to TGF-beta binding protein or variant or fragment thereof. See, e.g., U.S. Patent No. 5,223,409; Huse et al., 1989 Science 246:1275-81; Sastry et al., Proc. Natl. Acad. Sci. USA 86:5728-32 (1989); Altling-Mees et al., Strategies in Molecular Biology 3:1-9 (1990); Kang et al., 1991 Proc. Natl. Acad. Sci. USA 88:4363-66; Hoogenboom et al., 1992 J. Molec. Biol. 227:381-388; Schlebusch et al., 1997 Hybridoma 16:47-52 and references cited therein. For example, a library containing a plurality of polynucleotide sequences encoding Ig variable region fragments may be inserted into the genome of a filamentous bacteriophage, such as M13 or a variant thereof, in frame with the sequence encoding a phage coat protein. A fusion protein may be a fusion of the coat protein with the light chain variable region domain and/or with the heavy chain variable region domain. According to certain embodiments, immunoglobulin Fab fragments may also be displayed on a phage particle (see, e.g., U.S. Patent No. 5,698,426).

[0169] Heavy and light chain immunoglobulin cDNA expression libraries may also be prepared in lambda phage, for example, using λ ImmunoZap™(H) and λ ImmunoZap™(L) vectors (Stratagene, La Jolla, California). Briefly, mRNA is isolated from a B-cell population, and used to create heavy and light chain immunoglobulin cDNA expression libraries in the λ ImmunoZap(H) and λ ImmunoZap(L) vectors. These vectors may be screened individually or co-expressed to form Fab fragments or antibodies (see Huse *et al.*, *supra*; see also Sastry *et al.*, *supra*). Positive plaques may subsequently be converted to a non-lytic plasmid that allows high level expression of monoclonal antibody fragments from *E. coli*.

[0170] In one embodiment, in a hybridoma the variable regions of a gene expressing a monoclonal antibody of interest are amplified using nucleotide primers. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. (See, e.g., Stratagene (La Jolla, California), which sells primers for mouse and human variable regions including, among others, primers for V_{Ha} , V_{Hb} , V_{Hc} , V_{Hd} , C_{H1} , V_L and C_L regions.) These primers may be used to amplify heavy or light chain variable regions, which may then be inserted into vectors such as ImmunoZAP™H or ImmunoZAP™L (Stratagene), respectively. These vectors may then be introduced into *E. coli*, yeast, or mammalian-based systems for expression. Large amounts of a single-chain protein containing a fusion of the V_H and V_L domains may be produced using these methods (see Bird et al., Science 242:423-426, 1988).

[0171] Once cells producing antibodies according to the invention have been obtained using any of the above-described immunization and other techniques, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA therefrom according to standard procedures as described herein. The antibodies produced therefrom may be sequenced and the CDRs identified and the DNA coding for the CDRs may be manipulated as described previously to generate other antibodies according to the invention.

[0172] Activin A binding agents of the present invention preferably modulate activin A function in the cell-based assay described herein and/or the *in vivo* assay described herein and/or bind to one or more of the cysteine knot domains described herein and/or cross-block the binding of one of the antibodies described in this application and/or are cross-blocked from binding activin A by one of the antibodies described in this application. Accordingly such binding agents can be identified using the assays described herein.

[0173] In certain embodiments, antibodies are generated by first identifying antibodies that bind to one more of the cysteine knot domains provided herein and/or neutralize in the cell-based and/or *in vivo* assays described herein and/or cross-block the antibodies described in this application and/or are cross-blocked from binding activin A by one of the antibodies described in this application. The CDR regions from these antibodies are then used to insert into appropriate biocompatible frameworks to generate activin A binding agents. The non-CDR portion of the binding agent may be composed of amino acids, or may be a non-protein molecule. The assays described herein allow the characterization of binding agents. Preferably the binding agents of the present invention are antibodies as defined herein.

[0174] It will be understood by one skilled in the art that some proteins, such as antibodies, may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to

express the protein as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, R.J. Journal of Chromatography 705:129-134, 1995).

Nucleic acids

[0175] In one aspect, the present invention provides isolated nucleic acid molecules. The nucleic acids comprise, for example, polynucleotides that encode all or part of an antigen binding protein, for example, one or both chains of an antibody of the invention, or a fragment, derivative, mutein, or variant thereof, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector. The nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides, and artificial variants thereof (e.g., peptide nucleic acids).

[0176] Nucleic acids encoding antibody polypeptides (e.g., heavy or light chain, variable domain only, or full length) may be isolated from B-cells of mice that have been immunized with activin A. The nucleic acid may be isolated by conventional procedures such as polymerase chain reaction (PCR).

[0177] Nucleic acid sequences encoding the variable regions of the heavy and light chain variable regions are shown above. The skilled artisan will appreciate that, due to the degeneracy of the genetic code, each of the polypeptide sequences disclosed herein is encoded by a large number of other nucleic acid sequences. The present invention provides each degenerate nucleotide sequence encoding each antigen binding protein of the invention.

[0178] The invention further provides nucleic acids that hybridize to other nucleic acids (e.g., nucleic acids comprising a nucleotide sequence of any of A1-A14) under particular hybridization conditions. Methods for hybridizing nucleic acids are well-known in the art. See, e.g., Curr. Prot. in Mol. Biol., John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, a moderately stringent hybridization condition uses a prewashing solution containing 5X sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6X SSC, and a hybridization temperature of 55° C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42° C), and washing conditions of 60° C, in 0.5X SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6X SSC at 45° C, followed by one or more washes in 0.1X SSC, 0.2% SDS at 68° C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65, 70, 75, 80, 85, 90, 95, 98 or 99% identical to each other typically remain hybridized to each other. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and Curr. Prot. in Mol. Biol. 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA.

[0179] Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (e.g., an antigen binding protein) that it encodes. Mutations can be introduced using any technique known in the art. In one embodiment, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another embodiment, one or more randomly selected residues is changed using, for example, a random mutagenesis protocol. However it is made, a mutant polypeptide can be expressed and screened for a desired property (e.g., binding to activin A).

[0180] Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-essential amino acid residues. In one embodiment, a nucleotide sequence provided herein for A1-A14, or a desired fragment, variant, or derivative thereof, is mutated such that it encodes an amino acid sequence comprising one or more deletions or substitutions of amino acid residues that are shown herein for A1-A14 to be residues where two or more sequences differ. As described herein *inter alia*, A1-A14 refers to 14 sequences, A1, and A14, as well as the 12 intervening amino acid residues. In another embodiment, the mutagenesis inserts an amino acid adjacent to one or more amino acid residues shown herein for A1-A14 to be residues where two or more sequences differ. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively change the biological activity (e.g., binding of activin A) of a polypeptide that it encodes. For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative changes include changing the antigen specificity of an antigen binding protein.

[0181] In another aspect, the present invention provides nucleic acid molecules that are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences of the invention. A nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide of the invention, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion (e.g., an activin A binding portion) of a polypeptide of the invention.

[0182] Probes based on the sequence of a nucleic acid of the invention can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide of the invention. The probe can comprise a label group, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide.

[0183] In another aspect, the present invention provides vectors comprising a nucleic acid encoding a polypeptide of the invention or a portion thereof. Examples of vectors include, but are not limited to, plasmids, viral vectors, non-episomal mammalian vectors and expression vectors, for example, recombinant expression vectors.

[0184] The recombinant expression vectors of the invention can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. The recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells (e.g., SV40 early gene enhancer, Rous sarcoma virus promoter and cytomegalovirus promoter), those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences, see Voss et al., 1986, Trends Biochem. Sci. 11:287, Maniatis et al., 1987, Science 236:1237, incorporated by reference herein in their entireties), and those that direct inducible expression of a nucleotide sequence in response to particular treatment or condition (e.g., the metallothionin promoter in mammalian cells and the tet-responsive and/or streptomycin responsive promoter in both prokaryotic and eukaryotic systems (see *id.*). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

[0185] In another aspect, the present invention provides host cells into which a recombinant expression vector of the invention has been introduced. A host cell can be any prokaryotic cell (for example, *E. coli*) or eukaryotic cell (for example, yeast, insect, or mammalian cells (e.g., CHO cells)). Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods.

Indications

[0186] In one aspect, the present invention provides methods of treating a subject. The method can, for example, have a generally beneficial effect on the subject's health, e.g., it can increase the subject's expected longevity. Alternatively, the method can, for example, treat, prevent, cure, relieve, or ameliorate ("treat") a disease, disorder, condition, or illness ("a condition"). Among the conditions to be treated in accordance with the present invention are conditions characterized by inappropriate expression or activity of activin A. In some such conditions, the expression or activity level is too high, and the treatment comprises administering an activin A antagonist as described herein.

[0187] One example of a type of condition that can be treated using the methods and compositions of the present invention is a condition that involves cell growth, for example, a cancerous condition which is accompanied by cachexia. Thus, in one embodiment, the present invention provides compositions and methods for treating a cancerous condition. In particular, the cancerous condition is a gonadal cancer, including tumors of the ovary and testis. (Fujii, Y. et al., Am. J. Phys. Endocrin. Metab., 286:E927-E931, 2004; Reis, F. M. et al., J. Clin. Endocrin. 87:2277-2282, 2005.) Activin A is known for its action in stimulating FSH biosynthesis and secretion in the pituitary gland, and has a physiological role in the regulation of gonadal function. Activin A has been associated with many types of human cancers and in particular with tumors of the reproductive system. Specifically, overexpression or deregulation of activin A has been implicated in ovarian cancer, (Menon U, et al., BJOG: An International Journal of Obstetrics & Gynaecology; 107(9):1069-74, 2000. Choi KC, et al., Molecular & Cellular Endocrinology. 174(1-2):99-110, 2001; Zheng W, et al., American Journal of Reproductive Immunology. 44(2):104-13, 2000; Lambert-Messerlian GM, et al., Gynecologic Oncology. 74(1):93-7, 1999; Steller MD, et al., Molecular Cancer Research: MCR. 3(1):50-61, 2005; Corbellis L., et al., Journal of the Society for Gynecologic Investigation. 11(4):203-6, 2004; Welt CK, et al., Journal of Clinical Endocrinology & Metabolism.

82(11):3720-7, 1997; and Harada K., et al., *Journal of Clinical Endocrinology & Metabolism*. 81(6):2125-30, 1996, endometrial adenocarcinoma Otani, T, et al., *Gynecologic Oncology*. 83(1):31-8, 2001; Tanaka T, et al., *International Journal of Oncology*. 23(3):657-63, 2003 and prostate cancer (Thomas TZ, et al., *Journal of Clinical Endocrinology & Metabolism*. 82(11):3851-8, 1997; Zhang, Z, et al., *Biochemical & Biophysical Research Communications*. 234(2):362-5, 1997; and Risbridger GP, et al., *Molecular & Cellular Endocrinology*. 180(1-2):149-53, 2001

[0188] The cancerous condition can be any cancerous condition that can be treated using the compositions comprised herein, for example, activin A antigen binding proteins such as anti-activin A antibodies, antibody fragments, or antibody derivatives. Examples of cancerous conditions include, for example, acute lymphoblastic leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, childhood cerebellar astrocytoma, childhood cerebral astrocytoma, basal cell carcinoma, extrahepatic bile duct cancer, bladder cancer, osteosarcoma/malignant fibrous histiocytoma bone cancer, brain tumors (e.g., brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma), breast cancer, bronchial adenomas/carcinoids, Burkitt's Lymphoma, carcinoid tumor, gastrointestinal carcinoid tumor, carcinoma of unknown primary, primary central nervous system, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, cutaneous t-cell lymphoma, endometrial cancer, ependymoma, esophageal cancer, ewing's family of tumors, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, intraocular melanoma eye cancer, retinoblastoma eye cancer, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, germ cell tumors (e.g., extracranial, extragonadal, and ovarian), gestational trophoblastic tumor, glioma (e.g., adult, childhood brain stem, childhood cerebral astrocytoma, childhood visual pathway and hypothalamic), hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Hodgkin's lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, islet cell carcinoma (endocrine pancreas), Kaposi's Sarcoma, kidney (renal cell) cancer, laryngeal cancer, leukemia (e.g., acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myelogenous, and hairy cell), lip and oral cavity cancer, liver cancer, non-small cell lung cancer, small cell lung cancer, lymphoma (e.g., AIDS-related, Burkitt's, cutaneous t-cell, Hodgkin's, non-Hodgkin's, and primary central nervous system), Waldenstrom's Macroglobulinemia, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanoma, intraocular (eye) melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia, chronic myeloid leukemia, multiple myeloma, chronic myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, islet cell pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pheochromocytoma, pineoblastoma, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, non-melanoma skin cancer, merkel cell skin carcinoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, cutaneous t-cell lymphoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, gestational trophoblastic tumor, carcinoma of unknown primary site, cancer of unknown primary site, urethral cancer, endometrial uterine cancer, uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma, vulvar cancer, Waldenstrom's Macroglobulinemia, and Wilms' Tumor.

[0189] An oligopeptide or polypeptide is within the scope of the invention if it has an amino acid sequence that is at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to least one of the CDR's of antibodies A1-A14; and/or to a CDR of a activin A binding agent that cross-blocks the binding of at least one of antibodies A1-A14 to activin A, and/or is cross-blocked from binding to activin A by at least one of antibodies A1-A14; and/or to a CDR of a activin A binding agent wherein the binding agent can block the binding of activin A to activin A receptor.

[0190] Activin A binding agent polypeptides and antibodies are within the scope of the invention if they have amino acid sequences that are at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a variable region of at least one of antibodies A1-A14, and cross-block the binding of at least one of antibodies A1-A14 to activin A, and/or are cross-blocked from binding to activin A by at least one of antibodies A1-A14; and/or can block the inhibitory effect of activin A on an activin A receptor.

[0191] Therapeutic antibodies may be used that specifically bind to intact activin A, in which sequences in the region of approximately C11-S33 (first loop) and approximately C81-E111 (second loop) retain the conformation of native activin A. Such mapping and binding is described in Example 6, below.

[0192] Antibodies according to the invention may have a binding affinity for human activin A of less than or equal to $1 \times 10^{-7}M$, less than or equal to $1 \times 10^{-8}M$, less than or equal to $1 \times 10^{-9}M$, less than or equal to $1 \times 10^{-10}M$, less than

or equal to 1×10^{-11} M, or less than or equal to 1×10^{-12} M.

[0193] The affinity of an antibody or binding partner, as well as the extent to which an antibody inhibits binding, can be determined by one of ordinary skill in the art using conventional techniques, for example those described by Scatchard et al. (Ann. N.Y. Acad. Sci. 51:660-672 (1949)) or by surface plasmon resonance (SPR; BIACore, Biosensor, Piscataway, NJ). For surface plasmon resonance, target molecules are immobilized on a solid phase and exposed to ligands in a mobile phase running along a flow cell. If ligand binding to the immobilized target occurs, the local refractive index changes, leading to a change in SPR angle, which can be monitored in real time by detecting changes in the intensity of the reflected light. The rates of change of the SPR signal can be analyzed to yield apparent rate constants for the association and dissociation phases of the binding reaction. The ratio of these values gives the apparent equilibrium constant (affinity) (see, e.g., Wolff et al., Cancer Res. 53:2560-65 (1993)).

[0194] An antibody according to the present invention may belong to any immunoglobulin class, for example IgG, IgE, IgM, IgD, or IgA. It may be obtained from or derived from an animal, for example, fowl (e.g., chicken) and mammals, which includes but is not limited to a mouse, rat, hamster, rabbit, or other rodent, cow, horse, sheep, goat, camel, human, or other primate. The antibody may be an internalizing antibody. Production of antibodies is disclosed generally in U.S. Patent Publication No. 2004/0146888 A1.

Characterization Assays

[0195] In the methods described above to generate antibodies according to the invention, including the manipulation of the specific A1-A14 CDRs into new frameworks and/or constant regions, appropriate assays are available to select the desired antibodies (i.e. assays for determining binding affinity to activin A; cross-blocking assays; Biacore-based competition binding assay;" *in vivo* assays).

Therapeutic methods and administration of antigen binding proteins

[0196] Certain methods provided herein comprise administering an activin A binding antigen binding protein to a subject, thereby reducing an activin A-induced biological response that plays a role in a particular condition. In particular embodiments, methods of the invention involve contacting endogenous activin A with an activin A binding antigen binding protein, e.g., via administration to a subject or in an *ex vivo* procedure.

[0197] The term "treatment" encompasses alleviation or prevention of at least one symptom or other aspect of a disorder, or reduction of disease severity, and the like. In addition, "treatment" further relates to administering a therapeutic agent described herein for preventing or alleviating at least one symptom or other aspect of a disorder in a subject in need thereof. An antigen binding protein need not effect a complete cure, or eradicate every symptom or manifestation of a disease, to constitute a viable therapeutic agent. As is recognized in the pertinent field, drugs employed as therapeutic agents may reduce the severity of a given disease state, but need not abolish every manifestation of the disease to be regarded as useful therapeutic agents. Similarly, a prophylactically administered treatment need not be completely effective in preventing the onset of a condition in order to constitute a viable prophylactic agent. Simply reducing the impact of a disease (for example, by reducing the number or severity of its symptoms, or by increasing the effectiveness of another treatment, or by producing another beneficial effect), or reducing the likelihood that the disease will occur or worsen in a subject, is sufficient. One embodiment of the invention is directed to a method comprising administering to a patient an activin A antagonist in an amount and for a time sufficient to induce a sustained improvement over baseline of an indicator that reflects the severity of the particular disorder.

[0198] As is understood in the pertinent field, pharmaceutical compositions comprising the molecules of the invention are administered to a subject in need thereof in a manner appropriate to the indication. Pharmaceutical compositions may be administered by any suitable technique, including but not limited to parenterally, topically, or by inhalation. If injected, the pharmaceutical composition can be administered, for example, via intra-articular, intravenous, intramuscular, intralesional, intraperitoneal or subcutaneous routes, by bolus injection, or continuous infusion. Localized administration, e.g. at a site of disease or injury is contemplated, as are transdermal delivery and sustained release from implants. Delivery by inhalation includes, for example, nasal or oral inhalation, use of a nebulizer, inhalation of the antagonist in aerosol form, and the like. Other alternatives include eyedrops; oral preparations including pills, syrups, lozenges or chewing gum; and topical preparations such as lotions, gels, sprays, and ointments.

[0199] Use of antigen binding proteins in *ex vivo* procedures also is contemplated. For example, a patient's blood or other bodily fluid may be contacted with an antigen binding protein that binds full-length activin A, one or more activin A isoform, or other partial length activin A *ex vivo*. The antigen binding protein may be bound to a suitable insoluble matrix or solid support material.

[0200] Advantageously, antigen binding proteins are administered in the form of a composition comprising one or more additional components such as a physiologically acceptable carrier, excipient or diluent. Optionally, the composition additionally comprises one or more physiologically active agents, for example, a second activin A receptor-inhibiting

substance, an anti-angiogenic substance, a chemotherapeutic substance, an analgesic substance, *etc.*, non-exclusive examples of which are provided herein. In various particular embodiments, the composition comprises one, two, three, four, five, or six physiologically active agents in addition to an activin A-binding antigen binding protein

[0201] In one embodiment, the pharmaceutical composition comprise an antigen binding protein of the invention together with one or more substances selected from the group consisting of a buffer, an antioxidant such as ascorbic acid, a low molecular weight polypeptide (such as those having fewer than 10 amino acids), a protein, an amino acid, a carbohydrate such as glucose, sucrose or dextrans, a chelating agent such as EDTA, glutathione, a stabilizer, and an excipient. Neutral buffered saline or saline mixed with conspecific serum albumin are examples of appropriate diluents. In accordance with appropriate industry standards, preservatives such as benzyl alcohol may also be added. The composition may be formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Suitable components are nontoxic to recipients at the dosages and concentrations employed. Further examples of components that may be employed in pharmaceutical formulations are presented in Remington's Pharmaceutical Sciences, 16th Ed. (1980) and 20th Ed. (2000), Mack Publishing Company, Easton, PA.

[0202] Kits for use by medical practitioners include an antigen binding protein of the invention and a label or other instructions for use in treating any of the conditions discussed herein. In one embodiment, the kit includes a sterile preparation of one or more antigen binding proteins, which may be in the form of a composition as disclosed above, and may be in one or more vials.

[0203] Dosages and the frequency of administration may vary according to such factors as the route of administration, the particular antigen binding proteins employed, the nature and severity of the disease to be treated, whether the condition is acute or chronic, and the size and general condition of the subject. Appropriate dosages can be determined by procedures known in the pertinent art, e.g. in clinical trials that may involve dose escalation studies.

[0204] An antigen binding protein of the invention may be administered, for example, once or more than once, e.g., at regular intervals over a period of time. In particular embodiments, an antigen binding protein is administered over a period of at least a month or more, e.g., for one, two, or three months or even indefinitely. For treating chronic conditions, long-term treatment is generally most effective. However, for treating acute conditions, administration for shorter periods, e.g. from one to six weeks, may be sufficient. In general, the antigen binding protein is administered until the patient manifests a medically relevant degree of improvement over baseline for the chosen indicator or indicators.

[0205] Particular embodiments of the present invention involve administering an antigen binding protein at a dosage of from about 1 ng of antigen binding protein per kg of subject's weight per day ("1 ng/kg/day") to about 10 mg/kg/day, more preferably from about 500 ng/kg/day to about 5 mg/kg/day, and most preferably from about 5 µg/kg/day to about 2 mg/kg/day, to a subject. In additional embodiments, an antigen binding protein is administered to adults one time per week, two times per week, or three or more times per week, to treat an activin A mediated disease, condition or disorder, e.g., a medical disorder disclosed herein. If injected, the effective amount of antigen binding protein per adult dose may range from 1-20 mg/m², and preferably is about 5-12 mg/m². Alternatively, a flat dose may be administered; the amount may range from 5-100 mg/dose. One range for a flat dose is about 20-30 mg per dose. In one embodiment of the invention, a flat dose of 25 mg/dose is repeatedly administered by injection. If a route of administration other than injection is used, the dose is appropriately adjusted in accordance with standard medical practices. One example of a therapeutic regimen involves injecting a dose of about 20-30 mg of antigen binding protein one to three times per week over a period of at least three weeks, though treatment for longer periods may be necessary to induce the desired degree of improvement. For pediatric subjects (age 4-17), one exemplary suitable regimen involves the subcutaneous injection of 0.4 mg/kg, up to a maximum dose of 25 mg of antigen binding protein administered two or three times per week.

[0206] Particular embodiments of the methods provided herein involve subcutaneous injection of from 0.5 mg to 10 mg, preferably from 3 to 5 mg, of an antigen binding protein, once or twice per week. Another embodiment is directed to pulmonary administration (e.g., by nebulizer) of 3 or more mg of antigen binding protein once a week.

[0207] Examples of therapeutic regimens provided herein comprise subcutaneous injection of an antigen binding protein once a week, at a dose of 1.5 to 3 mg, to treat a condition in which activin A signaling plays a role. Examples of such conditions are provided herein and include, for example, cachexia, cancer, rheumatoid arthritis, and all conditions in which loss of body weight, body mass, body fat, or inability to maintain body weight, body mass, body fat, play a role. Weekly administration of antigen binding protein is continued until a desired result is achieved, e.g., the subject's symptoms subside. Treatment may resume as needed, or, alternatively, maintenance doses may be administered.

[0208] Other examples of therapeutic regimens provided herein comprise subcutaneous or intravenous administration of a dose of 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 15, or 20 milligrams of an activin A inhibitor of the present invention per kilogram body mass of the subject (mg/kg). The dose can be administered once to the subject, or more than once at a certain interval, for example, once a day, three times a week, twice a week, once a week, three times a month, twice a month, once a month, once every two months, once every three months, once every six months, or once a year. The duration of the treatment, and any changes to the dose and/or frequency of treatment, can be altered or varied during the course of treatment in order to meet the particular needs of the subject.

[0209] In another embodiment, an antigen binding protein is administered to the subject in an amount and for a time

sufficient to induce an improvement, preferably a sustained improvement, in at least one indicator that reflects the severity of the disorder that is being treated. Various indicators that reflect the extent of the subject's illness, disease or condition may be assessed for determining whether the amount and time of the treatment is sufficient. Such indicators include, for example, clinically recognized indicators of disease severity, symptoms, or manifestations of the disorder in question.

In one embodiment, an improvement is considered to be sustained if the subject exhibits the improvement on at least two occasions separated by two to four weeks. The degree of improvement generally is determined by a physician, who may make this determination based on signs, symptoms, biopsies, or other test results, and who may also employ questionnaires that are administered to the subject, such as quality-of-life questionnaires developed for a given disease.

[0210] A subject's levels of activin A may be monitored before, during and/or after treatment with an antigen binding protein, to detect changes, if any, in their levels. For some disorders, the incidence of elevated activin A levels may vary according to such factors as the stage of the disease or the particular form of the disease. Known techniques may be employed for measuring activin A levels, e.g., in a subject's serum. Activin A levels in blood samples may be measured using any suitable technique, for example, ELISA.

[0211] Particular embodiments of methods and compositions of the invention involve the use of an antigen binding protein and one or more additional activin A antagonists, for example, two or more antigen binding proteins of the invention, or an antigen binding protein of the invention and one or more other activin A antagonists. In further embodiments, antigen binding protein are administered alone or in combination with other agents useful for treating the condition with which the patient is afflicted. Examples of such agents include both proteinaceous and non-proteinaceous drugs. When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art. "Co-administration" and combination therapy are not limited to simultaneous administration, but also include treatment regimens in which an antigen binding protein is administered at least once during a course of treatment that involves administering at least one other therapeutic agent to the patient.

[0212] Examples of other agents that may be co-administered with an antigen binding protein are other antigen binding proteins or therapeutic polypeptides that are chosen according to the particular condition to be treated. Alternatively, non-proteinaceous drugs that are useful in treating one of the particular conditions discussed above may be co-administered with an activin A antagonist.

Combination therapy

[0213] In another aspect, the present invention provides a method of treating a subject with an activin A inhibiting antigen binding protein and one or more other treatments. In one embodiment, such a combination therapy achieves synergy or an additive effect by, for example, attacking multiple sites or molecular targets in a tumor. Types of combination therapies that can be used in connection with the present invention include inhibiting or activating (as appropriate) multiple nodes in a single disease-related pathway, multiple pathways in a target cell, and multiple cell types within a target tissue (e.g., within a tumor). For example, an activin A inhibitor of the present invention can be combined with a treatment that promotes apoptosis or inhibits angiogenesis. In another embodiment, a targeted agent, that, when used by itself, fails to elicit a therapeutically desired effect, could be used to, for example, sensitize cancer cells or augment treatment effect of other agents. In another embodiment, an activin A inhibitor according to the invention is used in combination with a cytotoxic drug or other targeted agent that induces apoptosis. In another embodiment, an activin A inhibitor is used in combination with one or more agents that inhibit different targets that are involved in cell survival (e.g., PKB, mTOR), different receptor tyrosine kinases (e.g., ErbB1, ErbB2, c-Met, c-kit), or different cell types (e.g., KDR inhibitors, c-fms). In another embodiment, an activin A inhibitor of the invention is added to the existing standard of care for a particular condition. Examples of therapeutic agents include, but are not limited to, gemcitabine, taxol, taxotere, and CPT-11.

[0214] In another embodiment, the method comprises administering one or more of the activin A antagonists described herein and one or more other treatments (e.g., a therapeutic or palliative treatment), for example, anti-cancer treatments (such as surgery, ultrasound, radiotherapy, chemotherapy, or treatment with another anti-cancer agent). Where a method comprises administering more than one treatment to a subject, it is to be understood that the order, timing, number, concentration, and volume of the administrations is limited only by the medical requirements and limitations of the treatment, i.e., two treatments can be administered to the subject, e.g., simultaneously, consecutively, alternately, or according to any other regimen. Examples of agents that can be administered in combination with the activin A antagonists described herein include, but are not limited to, neutrophil-boosting agents, irinotecan, SN-38, gemcitabine, herstatin, or an activin A-binding herstatin derivative (as described, for example, in U.S. Pat. App. No. 05/0272637), AVASTIN® (Genentech, South San Francisco, CA), HERCEPTIN® (Genentech), RITUXAN® (Genentech), ARIMIDEX® (AstraZeneca, Wilmington, DE), IRESSA® (AstraZeneca), BEXXAR® (Corixa, Seattle, WA), ZEVALIN® (Biogen Idec, Cambridge, MA), ERBITUX® (Imclone Systems Inc., New York, NY), GEMZAR® (Eli Lilly and Co., Indianapolis, IN), CAMPTOSAR® (Pfizer, New York, NY), GLEEVEC® (Novartis), SU-11248 (Pfizer), BMS-354825 (Bristol-Myers Squibb), panitumumab (Abgenix, Fremont, CA/Amgen Inc., Thousand Oaks, CA), and denosumab (Amgen Inc., Thousand Oaks,

CA).

[0215] The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., subcutaneous, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art, some of which are briefly discussed below for general purposes of illustration.

[0216] In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

[0217] In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein subcutaneously, parenterally, intravenously, intramuscularly, or even intraperitoneally. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U.S. Patent No. 5,543,158; U.S. Patent No. 5,641,515 and U.S. Patent No. 5,399,363. In certain embodiments, solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropyl-cellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally will contain a preservative to prevent the growth of microorganisms.

[0218] Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U.S. Patent No. 5,466,468). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0219] In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, Remington's Pharmaceutical Sciences, 15th ed., pp. 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. Moreover, for human administration, preparations will of course preferably meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

[0220] In another embodiment of the invention, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

[0221] The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

[0222] In certain embodiments, liposomes, nanocapsules, microparticles, lipid particles, vesicles, and the like, are used for the introduction of the compositions of the present invention into suitable host cells/organisms. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like. Alternatively, compositions of the present invention can be bound, either covalently or non-covalently, to the surface of such carrier vehicles.

[0223] The formation and use of liposome and liposome-like preparations as potential drug carriers is generally known to those of skill in the art (see for example, Lasic, Trends Biotechnol. 16(7):307-21, 1998; Takakura, Nippon Rinsho 56(3):691-95, 1998; Chandran et al., Indian J. Exp. Biol. 35(8):801-09, 1997; Margalit, Crit. Rev. Ther. Drug Carrier Syst. 12(2-3):233-61, 1995; U.S. Patent No. 5,567,434; U.S. Patent No. 5,552,157; U.S. Patent No. 5,565,213; U.S. Patent No. 5,738,868 and U.S. Patent No. 5,795,587, each specifically incorporated herein by reference in its entirety). The use of liposomes does not appear to be associated with autoimmune responses or unacceptable toxicity after systemic delivery. In certain embodiments, liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)).

[0224] Alternatively, in other embodiments, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (see, for example, Quintanar-Guerrero et al., Drug Dev. Ind. Pharm. 24(12): 1113-28, 1998). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μ m) may be designed using polymers able to be degraded *in vivo*. Such particles can be made as described, for example, by Couvreur et al., Crit. Rev. Ther. Drug Carrier Syst. 5(1):1-20, 1988; zur Muhlen et al., Eur. J. Pharm. Biopharm. 45(2):149-55, 1998; Zambaux et al., J. Controlled Release 50(1-3):31-40, 1998; and U.S. Patent No. 5,145,684.

[0225] In addition, pharmaceutical compositions of the present invention may be placed within containers, along with packaging material that provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) that may be necessary to reconstitute the pharmaceutical composition.

[0226] The dose administered may range from 0.01 mg/kg to 100 mg/kg of body weight. As will be evident to one of skill in the art, the amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth. Typically, the compositions may be administered by a variety of techniques, as noted above.

[0227] The invention also provides a diagnostic kit comprising at least one anti-activin A binding agent according to the present invention. The binding agent may be an antibody. In addition, such a kit may optionally comprise one or more of the following:

- (1) instructions for using the one or more binding agent(s) for screening, diagnosis, prognosis, therapeutic monitoring or any combination of these applications;
- (2) a labeled binding partner to the anti-activin A binding agent(s);
- (3) a solid phase (such as a reagent strip) upon which the anti-activin A binding agent(s) is immobilized; and
- (4) a label or insert indicating regulatory approval for screening, diagnostic, prognostic or therapeutic use or any combination thereof.

If no labeled binding partner to the binding agent(s) is provided, the binding agent(s) itself can be labeled with one or more of a detectable marker(s), e.g. a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

[0228] The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

RECOMBINANT EXPRESSION OF ACTIVIN A

[0229] Ultra filtration and diafiltration (UF/DF) of conditioned media. R-HuActivinA was expressed in chinese hamster ovary (CHO) cells. A series of steps was developed to generate active, purified material. The purification process began by concentrating (diafiltration) the conditioned media (C.M.) between 15 to 20 fold using an Amicon S10Y10 spiral cartridge. The media was then buffer exchanged (diafiltered) using 5 volumes of 10mM tris buffer Ph 7.0, filtered and stored.

[0230] Cation Exchange Chromatography. The r-HuActivin A was applied at 5 ml/minute at 4-80C to a 2.6 X 7 cm (Pharmacia) S-Sepharose Fast Flow column equilibrated in 10 mM Tris buffer Ph 7.0. The column was washed with the equilibration buffer. The r-HuActivin A was eluted from the column with a gradient of increasing sodium chloride concentration to 0.4M over 20 column volumes buffered with 10Mm Tris Ph 7.0. The appropriate fractions were pooled and stored 4-80C.

[0231] Reverse Phase HPLC C4 Chromatography. The S-Sepharose column pool pH was adjusted to 2.0 with trifluoroacetic acid (TFA). The pool was then applied to a 1 X 25 cm Vydac C4 column at room temperature equilibrated with 75% A buffer (0.1% TFA in water), and 25% B buffer (90% acetonitrile, 0.1% TFA, 9.9% water). The column was

then washed with the equilibration buffer. R-HuActivin A was eluted with a gradient of 25% B buffer to 50% B buffer over 50 minutes at a flow rate of 5 ml/minute. The appropriate fractions were pooled, and lyophilized.

[0232] High Performance Cation Exchange Chromatography. A 5ml S Sepharose High Performance column was equilibrated with 8M urea, 10 mM sodium phosphate, Ph 7.0 (A buffer) at room temperature. The lyophilized C4 pool was resuspended in A buffer and applied at 5 ml/minute, and washed with A buffer. The r-HuActivin A was eluted from the column with a gradient of increasing sodium chloride concentration to 0.15M over 30 column volumes buffered with 8M urea, 10 mM sodium phosphate, pH 7.0. The appropriate fractions were pooled and stored 4-80 °C.

[0233] Reverse Phase HPLC C4 Chromatography. The S-Sepharose column pool pH was adjusted to 2.0 with Trifluoroacetic Acid (TFA). The pool was then applied to a 1 X 25 cm Vydac C4 column at room temperature equilibrated with 75% A buffer (0.1% TFA in water), and 25% B buffer (90% acetonitrile, 0.1% TFA, 9.9% water). The column was then washed extensively to remove urea and salts with the equilibration buffer. R-HuActivin A was eluted with a gradient of 25%B buffer to 50% B buffer over 50 minutes at a flow rate of 5 ml/minute. The appropriate fractions were pooled, and the final purified r-HuActivin A was lyophilized and stored in aliquots -80 °C. SEQ ID NO:225 provides the amino acid sequence for activin A.

EXAMPLE 2

GENERATION OF ANTI-ACTIVIN A HYBRIDOMAS

[0234] Antibodies to activin A were raised in XenoMouse® mice (Abgenix, Fremont, Calif.), which are mice containing human immunoglobulin genes. The XenoMouse® strain XMG2 was used to produce fully human IgG2 Kappa antibodies. A second strain was used to produce fully human IgG4 Kappa antibodies. Mice were immunized with activin A.

[0235] The mice were injected with antigen (activin A) according to standard protocols (US 2005/0118643; WO 2005/694879) in the hind footpads (5 µg per footpad). Initial injections contained the adjuvant TiterMax® Gold (Sigma, Cat # T2684). In subsequent injections, each mouse was injected with a total of 5 µg of antigen in the adjuvant alum gel (aluminum phosphate gel adjuvant; Superfos Biosector a/s, distributed by E. M. Sargent Pulp and Chemical Co., Clifton N.J., cat # 1452-250). The final injection contained a total of 10 µg of antigen per mouse and did not contain an adjuvant.

[0236] Each mouse was bled two days after the sixth injection. Blood samples from those bleeds were assayed by ELISA to determine the titer of antibodies to activin A. Four days after the final injection, the mice were sacrificed and their draining lymph nodes were harvested and the lymphocytes were recovered. Lymphocytes from the mice of each of the three groups were separately pooled. To enrich the lymphocyte samples for B-cells, T-cells were depleted by adding anti-CD90 magnetic beads (Miltenyi Biotech cat. # 491-01) and then passing the lymphocytes through an LS⁺ column (Miltenyi Biotech cat. # 424-01).

[0237] Each of the samples of B-cell enriched lymphocytes was then fused with P3 myeloma cells using an electrocell fusion device (Genetronic, Inc., Model ECM 2001) to create hybridomas. The three groups of fused hybridoma lines were then plated in 96-well plates hybridoma media as described (WO 2005/094879) although other suitable media known in the art can be used. The hybridoma lines were cultured for 14 days at 37°C, in 15% CO₂.

[0238] After 14 days, culture supernatants were assayed by ELISA to detect the presence of human IgG antibodies to activin A. Culture supernatants that tested positive in that ELISA were tested for the presence of human kappa chain in a second ELISA. In that second ELISA, the conditions were identical to the first ELISA, except that the secondary antibody was a goat anti-human kappa chain antibody conjugated to horseradish peroxidase. Hybridomas that tested positive in both ELISA assays were further expanded to produce 5 ml of supernatant for subsequent testing.

[0239] A total of 160 anti-activin A hybridoma samples derived from the xeno mice were screened using a cell-based functional assay and BIAcore binding analysis as described in the Examples below. Twenty-three hybridomas were further characterized for their properties related to expression, purification, cell-based assay, binding analysis, sequence analysis, MS, and SEC. From these, three potent Mabs, A1, A2, and A3, were identified for further testing as described below. The amino acid sequences for these antibodies are as follows: A1: SEQ ID NO:9 (light chain variable); SEQ ID NO:84 (light chain constant); SEQ ID NO:10 (heavy chain variable); and SEQ ID NO:214 (heavy chain constant). A2: SEQ ID NO:25 (light chain variable); SEQ ID NO:100 (light chain constant); SEQ ID NO:26 (heavy chain variable); and SEQ ID NO:215 (heavy chain constant). A3: SEQ ID NO:41 (light chain variable); SEQ ID NO:108 (light chain constant); SEQ ID NO:42 (heavy chain variable); and SEQ ID NO:221 (heavy chain constant).

EXAMPLE 3

EXPRESSION AND PURIFICATION OF HUMAN ANTI-HUACTIVIN A ANTIBODIES IN CHO CELLS

[0240] CS-9 cells used for transfection of the anti-huActivin A expression plasmids were a serum-free suspension

CHO cell line. The CS-9 clone was selected as the host cell line for expression of recombinant proteins and banked in serum-free medium. The bank was tested for adventitious agents and sterility and found to be free of viral, mycoplasma and microbial agents.

[0241] Anti-hu Activin A expressing cell lines were scaled up using a typical fed-batch process. Cells were inoculated into a Wave bioreactor upon expansion. Culture was fed three times on approximately day 3, day 5 and day 9 with bolus feeds and harvested on day 11. Cells were spun down and conditioned media was filtered through a ten inch 0.45/0.2 micron pre filter, followed by a filtration through a six inch 0.2 micron filter.

Purification of Mab's from hybridoma conditioned media (C.M.):

[0242] To between 7 to 10 ml of C.M.'s was added 100 μ l of a 1:2 slurry of Mab Select resin equilibrated in PBS. The tubes were placed on rotators at 4-8°C overnight. The tubes were centrifuged at 1,000 X g for 5 minutes and the non-bound fraction was decanted. The resin was washed with 5ml of PBS, and centrifuged and decanted as above. The resin was then transferred to a SPIN-X, 0.45 μ m, 2ml tube. The resin was washed an additional two times with 0.5ml of PBS and centrifuged. The Mab's were eluted with 0.2ml of 0.1M acetic acid by incubating at room temperature with occasional mixing for 10 minutes. The tubes were centrifuged, and 30ul of 1M Tris buffer Ph 8.0 is added to the eluate. Purified Mab's were stored 4-8°C.

EXAMPLE 4

C2C12 CELL BASED ACTIVIN ACTIVITY ASSAY

[0243] This assay demonstrates the activin A neutralizing capability of the antibody being tested by measuring the extent that binding of activin A to its receptor is inhibited. An activin-responsive reporter cell line was generated by transfection of C2C12 myoblast cells (ATCC No: CRL-1772) with a pMARE-luc construct. The pMARE-luc construct was made by cloning twelve repeats of the CAGA sequence, representing the activin response elements (Dennler et al. EMBO 17: 3091-3100 (1998)) into a pLuc-MCS reporter vector (Stratagene cat # 219087) upstream of the TATA box. The myoblast C2C12 cells naturally express activin IIB receptors (actRIIB) on the cell surface. When activin binds the cell receptors, the Smad pathway is activated, and phosphorylated Smad binds to the response element (Macias-Silva et al. Cell 87:1215 (1996)), resulting in the expression of the luciferase gene. Luciferase activity is then measured using a commercial luciferase reporter assay kit (cat # E4550, Promega, Madison, WI) according to manufacturer's protocol.

[0244] A stable line of C2C12 cells that had been transfected with pMARE-luc (C2C12/pMARE clone #44) was used to measure activin activity according to the following procedure.

[0245] Equal numbers of the reporter cells (C2C12/pMARE clone #44) were plated into 96 well cultures. A first round screening using two dilutions of condition medium which contains antibodies was performed with the activin A concentration fixed at 4 nM. Recombinant mature activin A was pre-incubated for 1 hour at room temperature with condition medium at 2 x and 5 x dilutions respectively. The reporter cell culture was treated with activin with or without antibodies for six hours. Activin A activity was measured by determining the luciferase activity in the treated cultures. This assay was used to initially identify antibodies that inhibited the activin A signaling activity in the reporter assay. Subsequently, a nine point titration curve was generated with the activin A concentration fixed at 4 nM. The activin A was preincubated with each of the following nine concentrations of purified antibodies: 0.004 nM, 0.04 nM, 0.4 nM, 4 nM, 20 nM, 40 nM, 200 nM, 400 nM and 2 μ M for one hour before adding the mixture to the reporter cell culture. The IC₅₀ values were for a number of antibodies A1, A2 and A3 are provided in Table 3.

Table 3

MAb	Cell IC ₅₀ (nM)
A1	<3
A2	<3
A3	<3

EXAMPLE 5

BIAcore® ASSAY

[0246] An affinity analysis of activin A antibodies A1, A2 and A3 was performed on a BIAcore®3000 (Biacore, Inc., Piscataway, NJ), apparatus using sensor chip CM5, and 0.005 percent P20 surfactant (Biacore, Inc.) as running buffer. Recombinant mature activin A protein was immobilized to a research grade CM5 sensor chip (Biacore, Inc.) via primary amine groups using the Amine Coupling Kit (Biacore, Inc.) according to the manufacturer's suggested protocol.

[0247] Direct binding assays were used to screen antibodies in order of their ability to bind to immobilized activin A. Binding assays were carried by injection of two concentrations (40 and 400 nM) of each candidate antibody to the immobilized activin A surface at a flow rate of 50 µl/min for 3 minutes. After a dissociation time of 3 minutes, the surface was regenerated. Binding curves were compared qualitatively for binding signal intensity, as well as for dissociation rates. Antibody binding kinetic parameters including k_a (association rate constant), k_d (dissociation rate constant) and K_D (dissociation equilibrium constant) were determined using the BIA evaluation 3.1 computer program (Biacore, Inc.). The lower the dissociation equilibrium constants (expressed in nM), the greater the affinity of the antibody for activin A.

EXAMPLE 6

ACTIVIN A BINDING REGION MAPPING FOR MONOCLONAL ANTIBODIES

[0248] Antibody binding regions on activin A were determined using multiple biochemical methods, including western under reducing or non-reducing conditions, limited protease digestion using LysC, peptide analysis by MS, and peptide competition using BIAcore.

[0249] Cys-knots are key structural characteristics for TGF- β family members. Breaking S-S with reducing agent deteriorated activin A structure and decreased activin A binding to the neutralizing antibodies, including A-1. This data demonstrated that Cys-knots are important for activin A binding with these neutralizing antibodies that bind specifically to activin A compared to activin B. Cys-knots make two distant loops of sequences structurally adjacent to each other. These two regions are a sequence in the region of approximately C11-S33 (first loop) and approximately C81-E111 (second loop) activin A (Figure 7). A limited LysC digestion revealed that these two regions were protected by antibody A-1, indicating they interact with the neutralizing antibodies directly. The neutralizing antibodies are sensitive to conformational changes of activin A, suggesting they bind to non-linear epitopes of the antigen. Further peptide analysis indicated that fragments G-1 to K7 and S57-F74 in activin A are not required for its binding with the neutralizing antibodies directly. A comparison of the sequence of activin A compared to activin B shows that some of the sequence differences occur in this region.

EXAMPLE 7

SELECTIVITY ASSAYS

[0250] These assays were performed using BIAcore® technology, to determine the selectivity of binding of antibodies A1, A2 and A3 to various activins and other TGF- β family members, including activin A, activin B, activin AB, inhibin A, GDF-8, GDF-11, TGF- β -1, TGF- β -3, and BMP4 (all from R & D Systems). ActRIIB/Fc was covalently coupled to research grade sensor chips according to manufacturer's suggested protocol. Because BIAcore assays detect changes in the refractive index, the difference between the response detected with injection over the immobilized receptor surfaces compared with the response detected with injection over the control surface in the absence of any antibody represents the actual binding of the various ligands to the receptor. With pre-incubation of antibodies and activin and TGF- β molecules, a change (increase or decrease) in binding response indicates antibody binding to the TGF β family of molecules. The antibodies all bound to activin A but not to activin B, GDF-8, GDF-11, TGF- β -1, TGF- β -3, and BMP4, thus indicating specificity for activin A.

EXAMPLE 8

KINEX A™ EQUILIBRIUM ASSAYS

[0251] Solution-based equilibrium-binding assays using KinExA™ technology (Sapidyne Instruments, Inc.) were used to determine the dissociation equilibrium (K_D) of activin A binding to antibody molecules. This solution-based assay is considered to be more sensitive than the BIAcore assay in some instances. Reacti-Gel™ 6X was pre-coated with about 50 µg/ml activin A overnight, and then blocked with BSA. 30pM and 100pM of antibody samples were incubated with

various concentrations (0.5 pM to 5 nM) of activin A in sample buffer at room temperature for 8 hours before being run through the activin A-coated beads. The amount of the bead-bound antibody was quantified by fluorescent (Cy5) labeled goat anti-human-Fc antibody at 1 mg/ml in superblock. The binding signal was proportional to the concentration of free antibody at equilibrium with a given activin A concentration. KD was obtained from the nonlinear regression of the competition curves using a dual-curve one-site homogeneous binding model provided in the KinEx A™ software (Sapidyne Instruments, Inc.). The results are shown in Figure 8. A1 shows the strongest binding affinity for activin A ($K_D \sim 3$ pM). A2 and A3 bound to activin A at ~ 15 pM and ~ 8 pM, respectively.

EXAMPLE 9

PROTECTIVE EFFECTS OF ANTI-ACTIVIN ON BODY WEIGHT AND MUSCLE MASS LOSS IN COLLAGEN-INDUCED ARTHRITIS MODEL

[0252] This example was designed to test if activin inhibitors can rescue muscle wasting condition observed in collagen-induced arthritis. Collagen-induced arthritis (CIA) is a widely used mouse model sharing several clinical and pathological features with rheumatoid arthritis (RA). The precise mechanisms for CIA is not known, however, there is considerable evidence to suggest that CIA is a Th-1 mediated inflammatory disease. Rheumatoid arthritis is a common autoimmune disease that leads to joint inflammation, and progressive cartilage/ bone erosion. Even if the RA progression is under control, loss of BCM is not corrected without additional, direct intervention.

[0253] The collagen-induced arthritis model was prepared as follows. DBA/1J male mice (The Jackson Laboratory, Bar Harbor, ME), 8 weeks of age (20-23g), were used. Immunization was carried out on day 1 and day 21 by injecting 1000g Bovine Collagen II (Chondrex, Redmond, WA) emulsified in 100 μ l of CFA or ICFA, intradermally at the base of the tail. Three groups of ten mice each were used. Group 1 (control) received vehicle only. Group 2 (experimental, collagen injection) received vehicle only as treatment. Group 3 was injected with collagen and treated with anti-Activin A antibody A1.

[0254] The arthritic clinical index used was:

- 0=normal joint no signs of arthritis
- 1=swelling and/or redness of one digit
- 2=two joints involved
- 3= more than two joints
- 4= severe arthritis of the entire paw and digits

[0255] The treatment consisted of activin antibody injection (s.c.) beginning on Day 8, 5mg/kg, s.c. twice a week. The endpoints measured were body weight, muscle /fat mass, food intake and inflammatory cytokines.

[0256] Body weight. Untreated CIA animals lost twenty-five percent of their body weight and muscle mass compared to normal animals, which provided the evidence of rheumatoid cachexia. Anti-activin A (A-1) treatments significantly increased ($p < 0.05$) body weight compared to that of CIA control but not to the normal control animals.

[0257] Muscle mass. Treatment with monoclonal antibody A1 significantly increased muscle mass ($p < 0.05$) in CIA animals, compared to that of untreated CIA animals. In the CIA controls, the muscle mass at 95 days was 1.5g; whereas in the antibody-treated CIA animals, the muscle mass at 95 days was 2.5g. The results are shown in Figure 1.

[0258] Fat mass. Anti-activin A antibody did not reverse the fat loss in CIA animals. The results are shown in Figure 2.

[0259] The preservation of body weight and muscle mass in the treated animals provides support for activin A antibodies as a therapeutic for improving quality of life and lowering mortality in rheumatoid arthritis sufferers.

EXAMPLE 10

INTRAMUSCULAR CHO/ACTIVIN XENOGRAFT IN YOUNG ADULT NUDE MICE

[0260] CHO cells stably transfected with activin A (CHO/Activin) were implanted into young adult CD1 nu/nu mice via intramuscular injection at various doses, 1×10^6 , 5×10^6 , and 10×10^6 . The same doses of non-transfected CHO cells were injected into separate groups of mice as controls. CHO/Activin implantation induced a rapid and drastic body weight loss compared to controls.

[0261] At day 12, the CHO/control group for 1×10^6 cells had a loss of 10% of body weight, whereas the CHO/anti-activin A group had a 10% gain in body weight. The 5×10^6 and 10×10^6 cell anti-activin A groups also showed body weight increases of about 10%, whereas the control 5×10^6 and 10×10^6 cell groups lost 25-30% of the body weight.

[0262] Serum activin A levels in mice were measured on day 12 post xenograft implantation. The levels of serum activin A in parental CHO implanted control mice were < 2 ng/ml. In contrast, the mice bearing CHO/Activin xenograft

showed dramatically elevated serum activin A. There was a significant correlation between the serum activin A level and the severity of body weight loss as indicated by the statistical analysis, indicating that activin A overexpression is responsible for the body weight loss seen in CHO/Activin xenograft mice.

[0263] Mice (n=14 per group) were implanted with CHO/Activin xenograft and subsequently injected with either vehicle or each of the three anti-activin A monoclonal antibody, A1, A2 and A3. Twelve out of fourteen of the mice in the vehicle group died by day 25 post CHO/Activin implantation, while only one of forty-two CHO/Activin implanted mice in the anti-activin A Mab treatment groups died at the time. By day 38, the majority of mice in the Mab treatment groups continued to survive well, with survival rates as follows: thirteen out of fourteen for A1 group and ten out of fourteen for either A2 or A3 group.

[0264] Body weight data show that treatment with anti-activin A Mab, A1 or A2, completely prevented the body weight loss in CHO/Activin xenograft-bearing mice, indicating that the anti-activin A Mabs were effective in neutralizing activin A activity *in vivo*. As shown in Figure 3, NMR data revealed that treatment with anti-activin A Mab, A1 prevented the progressive loss of lean body mass seen in CHO/Activin-bearing mice. Treatment with this antibody also caused an increase in food intake.

[0265] Necropsy data indicate that treatment with anti-activin A Mab, A1 and A2, prevented the severe reduction in lean carcass weights seen in CHO/Activin-bearing mice (Table 5). Terminal necropsy data indicate that treatment with anti-activin A Mab, A1 and A2, prevented the severe reduction in fat mass seen in CHO/Activin-xenograft bearing mice (Table 5).

[0266] The percentage of animals bearing a visible tumor at the xenograft site was analyzed during terminal necropsy on day 12 post CHO/Activin implantation. As shown in Table 5, data revealed that 80% of the mice in the vehicle group developed visually identifiable xenograft tumors at the injection site. A significantly decreased rate of visible tumor formation in the anti-activin A Mab treatment groups was observed.

[0267] Upon necropsy, all the visually identifiable tumors at the CHO/Activin xenograft sites were dissected from the animals and weighed. A significant decrease in tumor mass was observed in the activin A Mab treatment group compared to vehicle group (Table 5).

Table 5

Treatment	Lean carcass mass (g) on day 12	Periuterine fat tissue (g) on day 12	Tumor xenograft development, percent of animals, on day 12	Tumor weight (g) on day 12
Nude mice plus vehicle	9 ± 0.25	0.18±0.02	Not applicable	Not applicable
CHO/Activin plus vehicle	7.5±0.25	0.10±0.02	80%	0.11±.01
CHO/Activin plus A-1 antibody	9.2±0.25	0.17±0.04	20%	0.01±0.005
CHO/Activin plus A-2 antibody	8.9±0.25	0.16±0.03	50%	0.01±0.005

[0268] The foregoing experiments on xenograft tumor development led to several conclusions regarding the use of anti-activin A antibodies to improve survival from cancer. Activin A played a causal role in the development of cachexia syndrome in nude mice bearing CHO/Activin xenograft. The loss of body weight correlated well with the increase in serum activin level in this model. Anti-activin A Mabs prevented the body weight loss and cachexia syndrome seen in CHO/Activin xenograft tumor-bearing mice. Anti-activin A Mabs suppressed xenograft growth, thereby significantly reducing the percentage of mice bearing visible xenograft tumors as well as decreasing the xenograft tumor sizes. Anti-activin A Mabs prevented the death resulting from CHO/Activin exograft, markedly promoting animal survival.

EXAMPLE 11

ANTI-ACTIVIN MONOCLONAL ANTIBODY A1 IN AAV-ACTIVIN MICE

[0269] Postnatal overexpression of activin A led to severe cachexia-like wasting syndrome in C57B1/6 mice. The body weight decreased over day 1, 4, 9 and 11, going from 16 grams to 14, 12.5, and finally 10.5 grams at day 11. There was

also a loss of fat weight, lean carcass weight, and gastrocnemius muscle weight.

[0270] To determine whether antibody directed to activin A could alleviate or prevent the effects of activin A, the following experiments were performed. AAV-Activin or empty AAV vector (control) were injected at 1×10^{13} pfu/mouse into 8 week old male C57B1/6 mice ($n=10-12$) via the tail veins. Figure 5 shows the effect of anti-Activin A monoclonal antibody A1 on body weight change in the transduced mice at days 1, 5, 8 and 12. The antibody prevented the body weight change observed in the control mice. Antibody treatment improved food intake in this animal model.

EXAMPLE 12

PROTECTIVE EFFECT OF ANI-ACTIVIN-A MAB A1 AGAINST BODY WEIGHT AND LEAN MASS LOSSES IN COLON-26 CANCER CACHEXIA MODEL

[0271] This example demonstrates the muscle preserving effect of the anti-Activin-A monoclonal antibody A-1 in a murine cancer cachexia model. The model of cancer cachexia was established by using the syngenic murine colon 26 adenocarcinoma cells inoculation in 8.5 weeks old male CDF1 mice (0.5×10^6 cells/mouse) on day 0. The anti-Activin-A MAb A-1 treatment (10 mg/kg, sc) was initiated on day 4 and was given 3 times weekly for 18 days. Sodium acetate buffer (10 mM sodium acetate, 5% sucrose, pH 5.0) as vehicle was used in the tumor-bearing control group mice. One group of age and weight matched normal CDF1 mice without tumors was used as parallel baseline control. Body weight and food intake were monitored three times weekly. Tumor size was measured three times per week by digital calipers. Body composition was measured using NMR at the beginning and the end of the study to monitor changes in lean and fat mass. At the end of the experiment, the mice were euthanized in a CO₂ chamber. Terminal block samples were collected and serum Activin-A levels were analyzed by ELISA. The lean carcass were weighed and recorded, and the gastrocnemius muscles and tumors were weighed and properly saved.

[0272] All results were expressed as mean $\pm$ standard error of the mean (SEM). Non-paired T-test was performed to determine statistical difference between groups by using the Graph Pad Prism software. Statistical significance from vehicle was represented by p values less than 0.05.

[0273] The data show that A-1 treated mice had significantly higher body weight than vehicle treated mice (21.30 ± 0.54 g vs. 19.21 ± 0.38 g, $P<0.05$, Day 15 and 19.66 ± 0.22 g vs. 18.11 ± 0.19 g, $P<0.05$, day 18). There was a significant body weight loss in tumor-bearing mice treated with vehicle compared to age-matched non-tumor-bearing mice (25.48 ± 0.35 g vs. 18.11 ± 0.19 g, $p<0.005$). Thus, the activin A antibody treatment helped to maintain body weight.

[0274] Tumor growth was monitored with a digitized calipers measurement and tumor size was calculated with the equation of: Tumor dimension ($\text{mm}^3 = L(\text{mm}) * W(\text{mm}) * W(\text{mm}) * 0.5$). The tumor size was not different between the antibody A1 treated and vehicle treated two groups.

[0275] After C-26 tumor cell inoculation, the mice in vehicle treated group had a dramatic body weight loss compared to non tumor-bearing mice (25.48 ± 0.35 g vs. 18.11 ± 0.19 g, $p<0.01$). However, A-1 treatment attenuated the average weight loss (19.66 ± 0.22 g vs. 18.11 ± 0.19 g, $P<0.05$).

[0276] A-1 treatment resulted in a significant preservation of the skeletal muscle mass. +A-1 treated mice had greater weight of the gastrocnemius muscle mass (0.099 ± 0.002 g vs. 0.093 ± 0.001 , $p<0.05$) than that in the C26 vehicle treated group.

[0277] At the end of the experiment, the terminal tissue dissection was performed. The control C-26 tumor-bearing mice had significantly lower lean carcass weight (6.75 ± 0.11 g) than that of the antibody A1 treated mice (7.20 ± 0.16 g, $p<0.05$ vs. C-26 + vehicle group).

[0278] The lean body mass was markedly lost in C-26 vehicle treated group (-1.85 ± 0.24 g compared to their initial body lean mass). Treatment with A-1 in C-26 tumor-bearing mice significantly attenuated the loss of lean body mass (-0.60 ± 0.26 g, $p<0.05$ vs. C26-vehicle group).

[0279] Anti-Activin A monoclonal antibody A1 treatment is effective in attenuation of cancer cachexia induced whole body weight lose. The protective effect of antibody A1 is associated with preserving skeletal muscle mass, lean carcass mass and total lean body mass in a well-established murine cancer cachexia animal model.

[0280] The present study provides preclinical evidence that neutralization of active A using monoclonal antibody A1 significantly attenuated body weight loss and preserved skeletal muscle and lean body mass.

EXAMPLE 13

ACTIVIN A ELISA

[0281] Antibodies to activin A can be used to assay and quantitate activin A in samples, such as biological samples. Recombinant human activin A (100 ng/ml, cat #10-85106) and assay diluting buffer (0 mg/ml, cat #10-85101) were purchased from DSLabs (Webster, Texas) and stored at 4°C. Standards were prepared fresh before the experiment by

diluting into assay buffer.

[0282] Human sera were obtained from Bioreclamation Inc. (Hicksville, NY). Sera for activin A measurement were aliquoted in 110 μ l to minimize variation due to freeze-thaw. The samples were diluted 1/3 with assay buffer and measured in the ELISA.

[0283] Activin A ELISA (one-step ELISA) is performed using the following steps:

Corning Costar 3590 96-well plates were coated with 100 μ l of 4 μ g/mL anti-activin A Mab (A2) overnight at room temperature while gently shaking at 500-600 rpm. The wells (400 μ l/well) were washed three times with PBS containing 0.02% (v/v) Tween 20. The wells were blocked in 300 μ l of I-blocking buffer for two hours at room temperature, then blocking buffer was removed.

[0284] 100 μ l of standard activin A / or 100 μ l of diluted samples were added, and 25 μ l of 0.5 μ g/mL anti-activin A mAb-HRP labelled (A1/HRP) was added in assay buffer. For free activin A measurements, sera were diluted in 1/3 with assay buffer. For total activin A measurements, sera were (1) acidified (pH 4-5) with 20% HCL (2 μ l per 110 μ l sera), (2) incubated for 15 minutes at room temperature, (3) neutralized by adding 2 μ l of 5 N NaOH (2 μ l per 110 μ l sera), and (4) diluted in 1/3 with assay buffer.

[0285] Incubation was carried out for 2 hours at room temperature while shaking at 600-700 rpm. The wells (400 μ l/well) were washed three times with PBS containing 0.02% (v/v) Tween-20. 100 μ l of TMB (R&D System, Minneapolis, MN) was added, followed by an incubation for twenty minutes at room temperature. 50 μ l of stop solution (R&D System, Minneapolis, MN) was added. OD measurement was performed using 450 nm in Molecular Device SpectraMax M5.

[0286] A standard curve was generated by plotting absorbance at 450 nm vs. the log of the rh-activin concentration, by using a log-log (or a five-parameter logistic) curve-fitting program of Molecular Device. Values for sample concentrations were obtained by interpolation of their absorbance from the standard.

[0287] The Capturing Antibody was A-3 anti-activin A mAb, 20.78 mg/ml. The Detection Antibody was A-1 anti-activin A mAb-HRP, 0.65 HRP/Ab, 12.05 mg/ml. The Blocking Buffer was I-blocking buffer. The wash buffer was PBS / 0.1% Tween-20. The assay buffer (Reagent Diluent) was 0 mg/ml activin A buffer.

EXAMPLE 14

ACTIVIN A PROTEASE PROTECTION ANALYSIS

[0288] Protease protection assays were conducted in order to identify epitope binding of activin A antibodies. Recombinant human activin A degraded preparation was analyzed by three methods. The first method examined an activin A preparation that had been degraded during purification. The second method included proteolysis of the activin A preparation with Lysine C, chymotrypsin, pepsin and thermolysin. The third method included chemical degradation of the preparation using cyanogen bromide.

[0289] Thus, for the proteolysis of human activin and antibody complex, 5 micrograms of activin A and 90 micrograms of antibody were mixed in 100 microliters of 0.1 M ammonium bicarbonate (pH 7.8) and kept at room temperature for approximately 20 minutes before treatment with 2% by weight of the particular selected protease. Digestion of the protein was allowed to proceed at 37 degrees Celsius for 90 minutes. Control samples containing activin A alone or antibody alone were carried out in an identical fashion. The samples were acidified prior to RP-HPLC analysis.

[0290] For the cyanogen bromide digestion of activin A, CNBr fragments of activin A were generated by incubating 10 micrograms of the protein with CNBr in 100 microliters of 90% TFA overnight at room temperature. The sample was kept in the dark throughout the incubation and was dried in a vacuum prior to RP-HPLC analysis.

[0291] The RP-HPLC was utilized to analyze the fragments generated as described above. Briefly, the column was equilibrated with solvent, the sample was injected and the column was washed with solvent before a linear gradient was applied. Column effluent was monitored by absorbance at 215 nm. The eluted samples were manually collected and analyzed by Edman degradation and mass spectrometry.

[0292] The preparation that had been degraded during purification contained the following species:

1. Gly¹-His⁵⁹ (6,456.2 Da)
2. Ser⁶⁰-Tyr⁹⁴ (4,102.9 Da)
3. Asp⁹⁵-Ser¹¹⁶ (2,452.8 Da)
4. Gly¹-Tyr⁹⁴ (10,541.1 Da)

[0293] The preparation that had been degraded by a chymotryptic-like activity had the following species cleaved at the locations indicated in SEQ ID NO: 225:

1. $_1\text{GLECDGKVNICCKKQFFVSFKDIGWNDWI}^{30}$
2. $_{31}\text{APSGYHANYCEGECPSHIAGTSGSSLSFH}\downarrow\text{S}_{60}$
3. $_{61}\text{TVINHYRMRGHSPFANLKSCCVPTKLRPMS}_{90}$
4. $_{91}\text{MLYY}\downarrow\text{DDGQNIKKDIQNMIVEECGCS}_{116}$

[0294] The species set forth in Figure 9 indicate the locations of cleavage sites when the activin A preparation was degraded by chymotrypsin, Lysine C (LysC), or Cyanogen Bromide (CNBr).

EXAMPLE 15

ACTIVIN A BINDING ASSAY

[0295] Monoclonal antibodies A1, A2, and A3 bind to activin A but not activin B, according to the binding affinities listed in Table 6. Thus, an affinity analysis of activin A antibodies A1, A2, and A3 was performed in order to determine the region or structure needed for neutralizing antibody binding. Several activin A binding proteins are known, including ActRII (A/B), ActRI (A/B)(Alt4), follistatin, and follistatin related gene (FLRG).

[0296] Binding assays were conducted to screen antibodies in order to assess their ability to bind to immobilized antibody surfaces (activin A and/or activin B). Binding assays were performed utilizing 2 nM rhActivin A and 20 nM of each antibody immobilized on a surface. The following antibodies were immobilized on a surface and tested: AKA1 (commercially available), AKA2 (commercially available), A1, and A3. Results of the antibody assay are indicated in Figure 10.

Anti-Activin A Ab	A2	A3	A1	AKA1	AKA2
Activin A	$K_D \sim 25 \text{ pM}$	$K_D \sim 11 \text{ pM}$	$K_D \sim 3 \text{ pM}$	$K_D \sim 4 \text{ pM}$	$K_D \sim 4 \text{ pM}$
Activin B	-----	-----	-----	-----	-----

EXAMPLE 16

ACTIVIN A BINDING ASSAY REGION MAPPING (BIAcore)

[0297] Blocking assays were conducted on immobilized recombinant human activin RIB-Fc chimera surfaces, recombinant human activin RIIB-Fc chimera surfaces, and recombinant human RIIA-Fc chimera recombinant surfaces, as described in Example 5. Monoclonal antibodies A1, A2, AKA1, and AKA2 on each chimera surface were incubated with 1 nM activin A, and the relative binding response (%) was measured. An increased binding response in the presence of antibodies indicates that activin A is able to bind to the immobilized receptor surfaces and the antibodies in solution simultaneously, which is referred to as "carry on". Results are indicated in Table 7 and Figure 11, where EC50 means the effective concentration that yields 50% binding.

TABLE 7

EC50 (nM)	rhActivin RIB/Fc Chimera	rhActivin RIIB/Fc Chimera	rhActivin FIIA/Fc Chimera
AKA1	Partially block	0.30	0.35
AKA2	Partially block	0.36	0.37
A1	0.29	0.29	0.29
A2	0.18	Carry on	Carry on

EXAMPLE 17

ACTIVIN A/B CHIMERAS

[0298] Activin A/B chimeras were generated in order to further assess the epitope binding abilities of monoclonal antibodies A1, A2, and A3, as described in Examples 5 and 16. As indicated in Figure 12, two chimeras were tested: Activin A 13/39 B (containing amino acids 1-116 of activin A except that amino acids at positions 13-39 of activin A are substituted with the corresponding amino acids at positions 13-39 from activin B-SEQ ID NO: 243), and activin A 82/107 B (containing amino acids 1-116 of activin A except that amino acids at positions 82-107 of activin A are substituted with the corresponding amino acids at positions 82-107 from activin B-SEQ ID NO: 244).

[0299] Briefly, a full length activin A clone was used as a template for amplification by PCR using Pfu ultra polymerase (Stratagene) and primers (SEQ ID NO: 245 and SEQ ID NO: 246) at the start of the mature protein sequence. The resulting PCR product was column purified (Qiagen), digested with Sall and XbaI restriction enzymes (Roche) and gel isolated (Qiagen). The synthetic gene cassettes containing the modified mature protein sequences were designed by utilizing the amino acid sequences from full length activin A and activin B and back translating into DNA sequences codon optimized for expression in a mammalian host cell by using the Gene Designer program (Version 1.0.4, DNA 2.0, Inc.) (BMC Bioinformatics, 7: 285 (2006)). The sequences were digested with XbaI and NotI and gel isolated. The activin A PCR product was ligated under standard reaction conditions using T4 DNA ligase (New England Biolabs, Inc.) with either the 13-39 synthetic gene fragment (SEQ ID NO: 247) or the 82-107 synthetic gene fragment (SEQ ID NO: 213) and a Sall/NotI digested expression vector (pDRSalpha 24) to produce full length expression constructs. The synthetic gene construct of activin A with amino acids 13-39 replaced with activin B sequence (SEQ ID NO: 247) and the synthetic gene construct of activin A with amino acids 82-107 replaced with activin B sequence (SEQ ID NO: 248) were then utilized in the epitope mapping experiments (results shown in Figure 12).

[0300] From the foregoing, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims. All publications, published patent applications, and patent documents disclosed herein are hereby incorporated by reference.

[0301] The invention comprises at least the following numbered embodiments.

1. An isolated antigen binding protein comprising either:

a. a light chain CDR3 comprising a sequence selected from the group consisting of:

i. a light chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence selected from the group consisting of the light chain CDR3 sequences of SEQ ID NO:13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, and 205;

ii. $X_{73} Q X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80}$;

iii. $L Q H N X_{81} Y X_{82} X_{83} T$; and

iv. $Q A W D X_{84} S T X_{85} X_{86}$; or

b. a heavy chain CDR3 comprising a sequence selected from the group consisting of:

i. a heavy chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence selected from the group consisting of the heavy chain CDR3 sequences of H1-H14 of SEQ ID NO:16, 32, 48, 64, 80, 96, 112, 144, 160, 176, 208, and 224;

ii. $X_{87} X_{88} X_{89} X_{90} X_{91} X_{92} X_{93} X_{94} F D Y$;

iii. $X_{95} X_{96} X_{97} Y X_{98} D X_{99} X_{100} G W X_{101} X_{102} X_{103}$;

iv. $X_{104} X_{105} X_{106} X_{107} X_{108} X_{109} Y X_{110} X_{111} X_{112} X_{113} X_{114} X_{115} X_{116} X_{117} X_{118}$;

or

c. the light chain CDR3 sequence of (a) and the heavy chain CDR3 sequence of (b);

wherein

X_{73} is a methionine residue, a glutamine residue, or an arginine residue,

X_{74} is an alanine residue, a tyrosine residue, a glutamine residue, or a serine residue,

X_{75} is a leucine residue, a tyrosine residue, or an asparagine residue,

X_{76} is a glutamine residue, a serine residue, or a threonine residue,

X_{77} is a threonine residue, a tyrosine residue, or an isoleucine residue,

X_{78} is a proline residue or a serine residue,

X₇₉ is a cysteine residue, a tryptophan residue, a leucine residue, or a proline residue,
X₈₀ is a serine residue or a threonine residue,
X₈₁ is a threonine residue or a serine residue,
X₈₂ is a proline residue or a threonine residue,
5 X₈₃ is a phenylalanine residue or a tryptophan residue,
X₈₄ is an arginine residue or a serine residue,
X₈₅ is a valine residue or an alanine residue,
X₈₆ is a valine residue or no residue,
X₈₇ is a valine residue or no residue,
10 X₈₈ is a glutamine residue or no residue,
X₈₉ is an aspartate residue, a tryptophan residue, or no residue,
X₉₀ is a serine residue, a leucine residue, or no residue,
X₉₁ is an isoleucine residue, a glutamate residue, or a glutamine residue,
X₉₂ is an alanine residue, a leucine residue, or a glycine residue,
15 X₉₃ is an alanine residue or a leucine residue,
X₉₄ is a proline residue, a tyrosine residue, or a glycine residue,
X₉₅ is an aspartate residue or no residue,
X₉₆ is a glutamine residue or no residue,
X₉₇ is an aspartate residue or an alanine residue,
20 X₉₈ is a tyrosine residue or a glycine residue,
X₉₉ is a serine residue or a tyrosine residue,
X₁₀₀ is a serine residue or an arginine residue,
X₁₀₁ is a phenylalanine residue or no residue,
X₁₀₂ is a glycine residue or an aspartate residue,
25 X₁₀₃ is a histidine residue or a proline residue,
X₁₀₄ is a glycine residue or no residue
X₁₀₅ is a serine residue, a glutamate residue, or no residue
X₁₀₆ is an arginine residue, a serine residue, or no residue,
X₁₀₇ is an aspartate residue, an asparagine residue, a serine residue, or a glutamine residue
30 X₁₀₈ is a serine residue, an arginine residue, or a tryptophan residue,
X₁₀₉ is a glycine residue, an aspartate residue, an asparagine residue, a tyrosine residue, or a leucine residue,
X₁₁₀ is a serine residue, a glycine residue, an aspartate residue, or no residue,
X₁₁₁ is a serine residue, a valine residue, an asparagine residue, or a tyrosine residue,
X₁₁₂ is a serine residue, an asparagine residue, a tyrosine residue, or a histidine residue
35 X₁₁₃ is a tryptophan residue, a tyrosine residue, or a glutamine residue,
X₁₁₄ is a histidine residue, an aspartate residue, a tyrosine residue, or no residue,
X₁₁₅ is a phenylalanine residue, an alanine residue, or a glycine residue,
X₁₁₆ an aspartate residue, a phenylalanine residue, a leucine residue, or a methionine residue
X₁₁₇ a tyrosine residue, or an aspartate residue,
40 X₁₁₈ is an isoleucine residue, a valine residue, or no residue.
and said antigen binding protein binds specifically to human activin A.

2. The isolated antigen binding protein of embodiment 1, comprising an amino acid sequence selected from the group consisting of:

- a. a light chain CDR1 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR1 sequence of L1-L14 of SEQ ID NO:11, 27, 43, 59, 75, 91, 107, 155, 171, 203, and 219;
- b. a light chain CDR2 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR2 sequence of L1-L14 of SEQ ID NO:12, 28, 44, 60, 76, 156, 172, 204, and 220;
- c. a light chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence of L1-L14 of SEQ ID NO:13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, and 205;
- d. a heavy chain CDR1 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR1 sequence of H1-H14 of SEQ ID NO:14, 30, 46, 62, 78, 94, 110, 126, 158, 174, 190, 206, and 222;
- e. a heavy chain CDR2 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR2 sequence of H1-H14 of SEQ ID NO:15, 31, 47, 63, 79, 95, 111, 127, 159, 175,

207, and 223; and

f. a heavy chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence of H1-H14 of SEQ ID NO:16, 22, 32, 48, 64, 80, 96, 112, 144, 160, 176, 192, 208, and 244.

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3. The isolated antigen binding protein of embodiment 1, comprising a sequence selected from the group consisting of:

a. a light chain CDR1 sequence selected from the group consisting of:

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- i. (R/K)SSQS(L/I)L(H/Y)S(T/S)(G/N)(Y/N)(N/K)(-/K)YL(D/V);
- ii. RA(S/G)QGI(S/R)N(D/N)L(V/G);
- iii. RASQISISNYLNT; and
- iv. SG(D/E)K(L/W)G(D/E)K(F/Y)(A/V)(F/C);

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b. a light chain CDR2 sequence selected from the group consisting of:

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- i. (H/Q/L)D(T/N/S)KRPS; and
- ii. $X_{40} X_{41} S X_{42} X_{43} X_{44} S$, wherein
 X_{40} is an alanine residue, a tryptophan residue, or a leucine residue,
 X_{41} is a threonine residue, an alanine residue, or a glycine residue,
 X_{42} is a serine residue, a methionine residue, or a phenylalanine residue,
 X_{43} is a leucine residue or an arginine residue,
 X_{44} is a glutamine residue, a glutamate residue, or an alanine residue,

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c. a heavy chain CDR1 sequence selected from the group consisting of:

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- i. GGS(I/F)(N/S)(S/A)(-/G)(-/G)(F/Y)YWS;
- ii. $G X_{27} X_{28} F X_{29} X_{30} Y X_{31} X_{32} X_{33}$, wherein
 X_{27} is a tyrosine residue or a phenylalanine residue,
 X_{28} is a threonine residue or a serine residue,
 X_{29} is a threonine residue, a serine residue, or an isoleucine residue,
 X_{30} is a glycine residue or a serine residue,
 X_{31} is a tyrosine residue, a glycine residue, or a tryptophan residue,
 X_{32} is an isoleucine residue or a methionine residue,
 X_{33} is a histidine residue or a glycine residue; and
- iii. G(Y/F)TF(T/S)(S/A)Y(G/W)(L/M/I)(S/H);

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d. a heavy chain CDR2 sequence selected from the group consisting of:

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- i. (Y/E)I(S/Y/N)(Y/H)SG(S/G)T(Y/N)YNPSLK(S/R);
- ii. (V/N)I(K/W)(Y/Q)DGS(N/E/T)(K/E)Y(H/Y)(A/V)DSVKG; and
- iii. $X_{60} I X_{61} X_{62} X_{63} X_{64} X_{65} X_{66} T X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} Q G$
 X_{60} is a tryptophan residue or an isoleucine residue,
 X_{61} is an asparagine residue, an isoleucine residue, a serine residue, or a tyrosine residue,
 X_{62} is a proline residue or an alanine residue,
 X_{63} is an asparagine residue, a tyrosine residue, or a glycine residue,
 X_{64} is a serine residue, an asparagine residue, or an aspartate residue,
 X_{65} is a glycine residue or a serine residue,
 X_{66} is a glycine residue, an asparagine residue, or an aspartate residue,
 X_{67} is an asparagine residue or an arginine residue,
 X_{68} is a tyrosine residue or a serine residue,
 X_{69} is an alanine residue or a serine residue,
 X_{70} is a glutamine residue or a proline residue,
 X_{71} is a lysine residue or a serine residue, and
 X_{72} is a phenylalanine residue or a leucine residue.

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wherein amino acid residue symbols enclosed in parentheses identify alternative residues for the same position in a sequence.

4. The isolated antigen binding protein of embodiment 1, comprising either:

a. a light chain variable domain comprising:

- i. a light chain CDR1 sequence of SEQ ID NO:11, 27, 43, 59, 75, 91, 107, 155, 171, 203, and 219;
- ii. a light chain CDR2 sequence of SEQ ID NO:12, 28, 44, 60, 76, 156, 172, 204, and 220; and
- iii. a light chain CDR3 sequence of SEQ ID NO:13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, and 205;

b. a heavy chain variable domain comprising:

- i. a heavy chain CDR1 sequence of SEQ ID NO:14, 30, 46, 62, 78, 94, 110, 126, 158, 174, 190, 206, and 222;
- ii. a heavy chain CDR2 sequence of SEQ ID NO:15, 31, 47, 63, 79, 95, 111, 127, 159, 175, 191, 207, and 223; and
- iii. a heavy chain CDR3 sequence of SEQ ID NO:16, 32, 48, 64, 80, 96, 112, 144, 160, 176, 208, and 224; or

c. the light chain variable domain of (a) and the heavy chain variable domain of (b).

5. An isolated antigen binding protein comprising either:

a. a light chain variable domain sequence selected from the group consisting of:

- i. a sequence of amino acids at least 80% identical to a light chain variable domain sequence of L1-L14 of SEQ ID NO:9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, and 217;
- ii. a sequence of amino acids encoded by a polynucleotide sequence that is at least 80% identical to a polynucleotide sequence encoding a light chain variable domain sequence of L1-L14 of SEQ ID NO:9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, and 217; and
- iii. a sequence of amino acids encoded by a polynucleotide sequence that hybridizes under moderately stringent conditions to the complement of a polynucleotide consisting of a light chain variable domain sequence of L1-L14 of SEQ ID NO:1, 17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, and 209;

b. a heavy chain variable domain sequence selected from the group consisting of:

- i. a sequence of amino acids at least 80% identical to a heavy chain variable domain sequence of H1-H14 of SEQ ID NO:10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, and 218;
- ii. a sequence of amino acids encoded by a polynucleotide sequence that is at least 80% identical to a polynucleotide sequence encoding a heavy chain variable domain sequence of H1-H14 of SEQ ID NO:10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, and 218; and
- iii. a sequence of amino acids encoded by a polynucleotide sequence that hybridizes under moderately stringent conditions to the complement of a polynucleotide consisting of a heavy chain variable domain sequence of H1-H14 of SEQ ID NO:2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, and 210

c. the light chain variable domain of (a) and the heavy chain variable domain of (b);

wherein said antigen binding protein binds to human activin A.

6. The isolated antigen binding protein of embodiment 5 comprising either:

- a. a light chain variable domain sequence selected from the group consisting of L1-L14 of SEQ ID NO:9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, and 217;
- b. a heavy chain variable domain sequence selected from the group consisting of H1-H14 of SEQ ID NO:10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, and 218; or
- c. the light chain variable domain of (a) and the heavy chain variable domain of (b).

7. The isolated antigen binding protein of embodiment 1 or embodiment 5 that, when bound to activin A:

- a. inhibits activin A;
- b. cross-competes with a reference antibody for binding to activin A;
- c. binds to the same epitope of activin A as said reference antibody;
- d. binds to activin A with substantially the same K_d as said reference antibody; or

e. binds to activin A with substantially the same off rate as said reference antibody;

wherein said reference antibody comprises a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14.

8. The isolated antigen binding protein of embodiment 1 or embodiment 5 that, when bound to a human activin A, inhibits binding of activin A to human activin A receptor.

9. The isolated antigen binding protein of embodiment 1 or embodiment 5 wherein said antigen binding protein possesses at least one *in vivo* biological activity of a human anti-Activin A antibody.

10. The isolated antigen binding protein of embodiment 9 wherein said biological activity is attenuation of cachexia.

11. The isolated antigen binding protein of embodiment 9, that attenuates cachexia in colon tumor-bearing mice.

12. The isolated antigen binding protein of embodiment 9, that ameliorates the loss of body weight in colon tumor-bearing mice.

13. The isolated antigen binding protein of embodiment 9, that ameliorates the loss of body weight in a collagen-induced animal model of rheumatoid arthritis.

14. The isolated antigen binding protein of embodiment 9, that ameliorates the loss of muscle mass in a collagen-induced animal model of rheumatoid arthritis.

15. The isolated antigen binding protein of embodiment 9, that ameliorates the loss of fat mass in a collagen-induced animal model of rheumatoid arthritis.

16. The isolated antigen binding protein of embodiment 9, that ameliorates the loss of body weight in a AAV-activin A transfected animal model.

17. An isolated fully human antibody that specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin A, wherein said antigen binding protein possesses at least one *in vivo* biological activity of a human anti-activin A antibody.

18. The isolated antibody of embodiment 17 wherein said biological activity is attenuation of cachexia.

19. The isolated antibody of embodiment 17, that attenuates cachexia in colon tumor-bearing mice.

20. The isolated antibody of embodiment 17, that ameliorates the loss of body weight in colon tumor-bearing mice.

21. The isolated antibody of embodiment 17, that ameliorates the loss of body weight in a collagen-induced animal model of rheumatoid arthritis.

22. The isolated antibody of embodiment 17, that ameliorates the loss of muscle mass in a collagen-induced animal model of rheumatoid arthritis.

23. The isolated antibody of embodiment 17, that ameliorates the loss of fat mass in a collagen-induced animal model of rheumatoid arthritis.

24. The isolated antibody of embodiment 17, that ameliorates the loss of body weight in a AAV-activin A transfected animal model.

25. A fully human antibody that specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin A, wherein said antigen binding protein inhibits the binding of activin A to activin A receptor *in vitro*.

26. A fully human isolated antibody that specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin A, wherein said antigen binding protein inhibits the binding of activin A to activin A receptor *in vivo*.

27. A fully human isolated antibody that specifically binds to amino acids K13-Y39 of activin A, wherein said antigen binding protein inhibits the binding of activin A to activin A receptor *in vitro*.

28. A fully human isolated antibody that specifically binds to amino acids V82-N107 of activin A, wherein said antigen binding protein inhibits the binding of activin A to activin A receptor *in vitro*.

29. A fully human isolated antibody that specifically binds to amino acids K13-Y39 of activin A, wherein said antigen binding protein inhibits the binding of activin A to activin A receptor *in vivo*.

30. A fully human isolated antibody that specifically binds to amino acids V82-N107 of activin A, wherein said antigen binding protein inhibits the binding of activin A to activin A receptor *in vivo*.

Claims

1. An isolated antibody, or an antigen binding fragment thereof, that specifically binds to a cysteine knot region of human activin A, said region spanning amino acids C11-S33 and amino acids C81-E111 of the sequence set forth in SEQ ID NO:225, and inhibits binding of human activin A to human activin A receptor

2. The isolated antibody or antigen binding fragment of claim 1, wherein said antibody or antigen binding fragment

possesses at least one *in vivo* biological activity selected from attenuation of cachexia, attenuating cachexia in colon tumor-bearing mice, ameliorating the loss of body weight in colon tumor-bearing mice, ameliorating the loss of body weight in a collagen-induced animal model of rheumatoid arthritis, ameliorating the loss of muscle mass in a collagen-induced animal model of rheumatoid arthritis, ameliorating the loss of fat mass in a collagen-induced animal model of rheumatoid arthritis, and ameliorating the loss of body weight in a AAV-activin A transfected animal model.

3. The isolated antibody or antigen binding fragment of claim 1 or 2, which is fully human.
4. The isolated antibody or antigen binding fragment according to any one of the preceding claims for use in therapy.
5. An isolated polynucleotide that encodes the isolated antibody or antigen binding fragment of any one of claims 1 to 3.
6. A vector comprising a nucleic acid encoding the polynucleotide according to claim 5.
7. The vector according to claim 6, which is selected from the group consisting of a plasmid, a viral vector, non-episomal mammalian vector, expression vector and a recombinant expression vector.
8. A host cell into which a recombinant expression vector according to claim 7 has been introduced.
9. The host cell according to claim 8, which is a prokaryotic or eukaryotic cell, *e.g.* a mammalian cell, optionally a Chinese Hamster Ovary (CHO) cell.
10. A pharmaceutical composition comprising an isolated antibody or antigen binding fragment according to any one of claims 1 to 3 together with one or more substances selected from the group consisting of a buffer, an antioxidant, a low molecular weight polypeptide, a protein, an amino acid, a carbohydrate, a chelating agent, a stabilizer, and an excipient.
11. A pharmaceutical composition comprising an isolated antibody or antigen binding fragment according to any one of claims 1 to 3 for use in therapy.

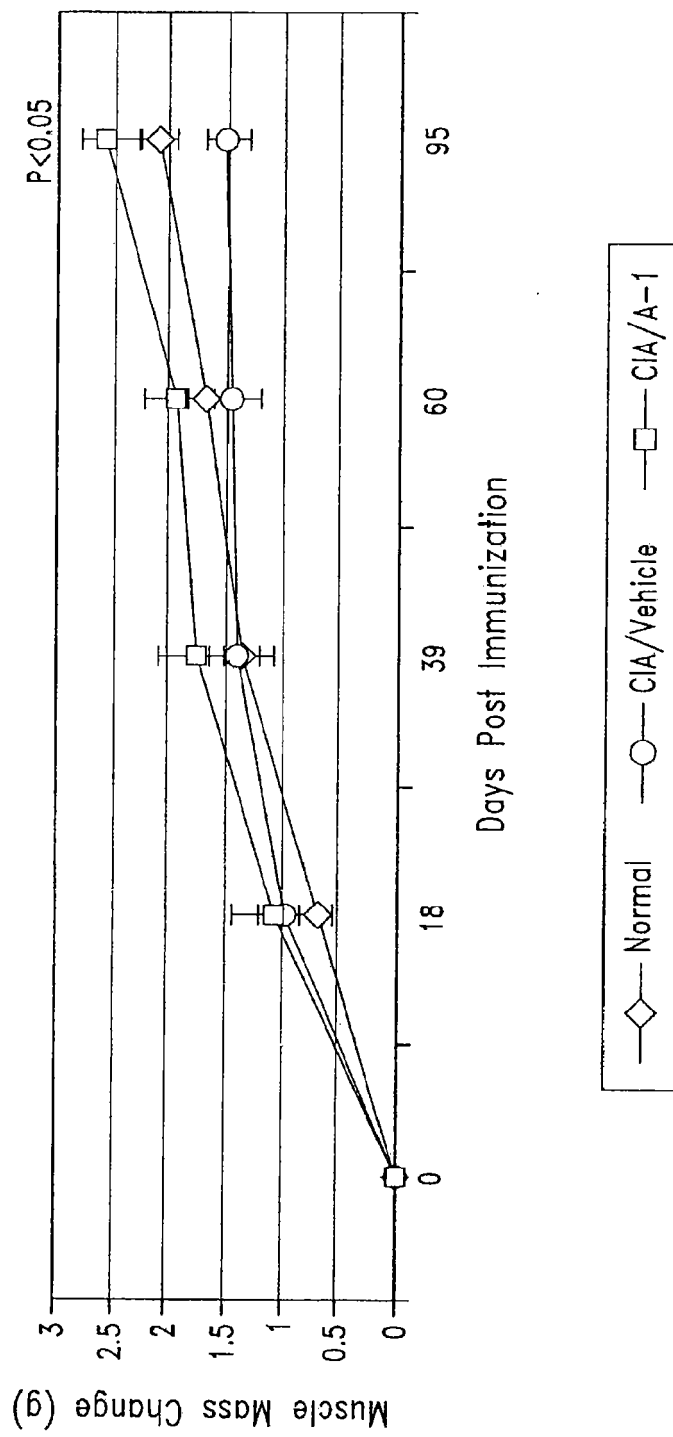
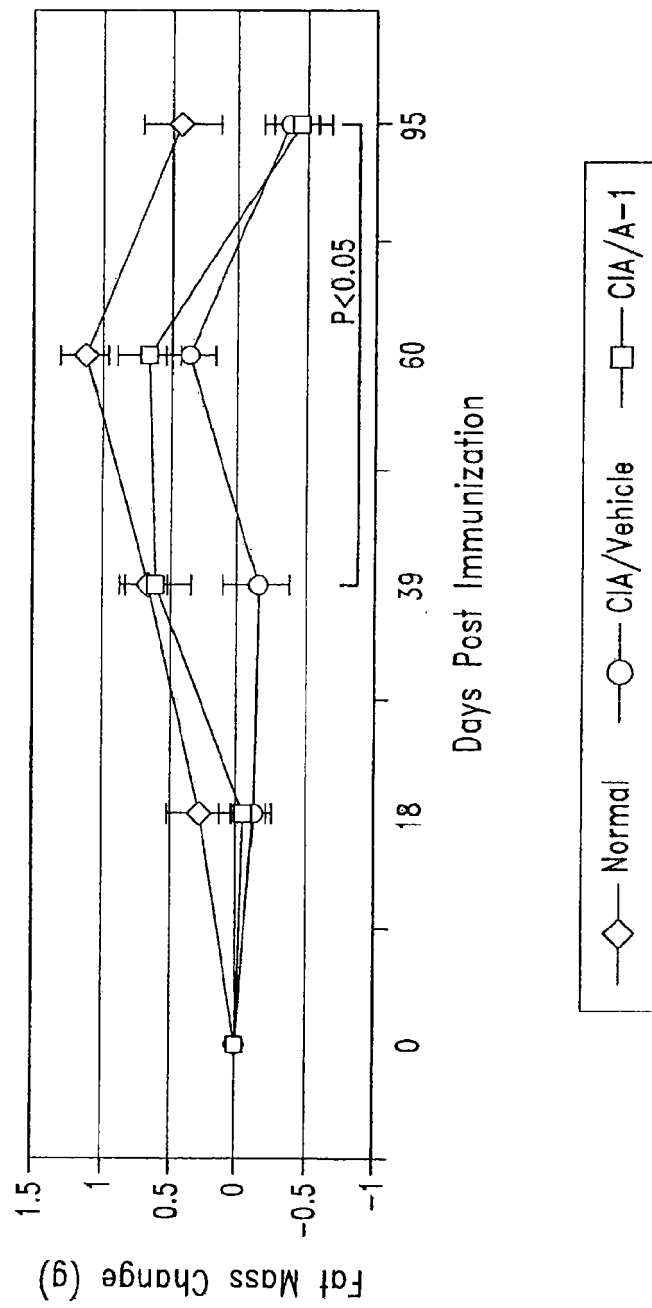


Fig. 1

*Fig. 2*

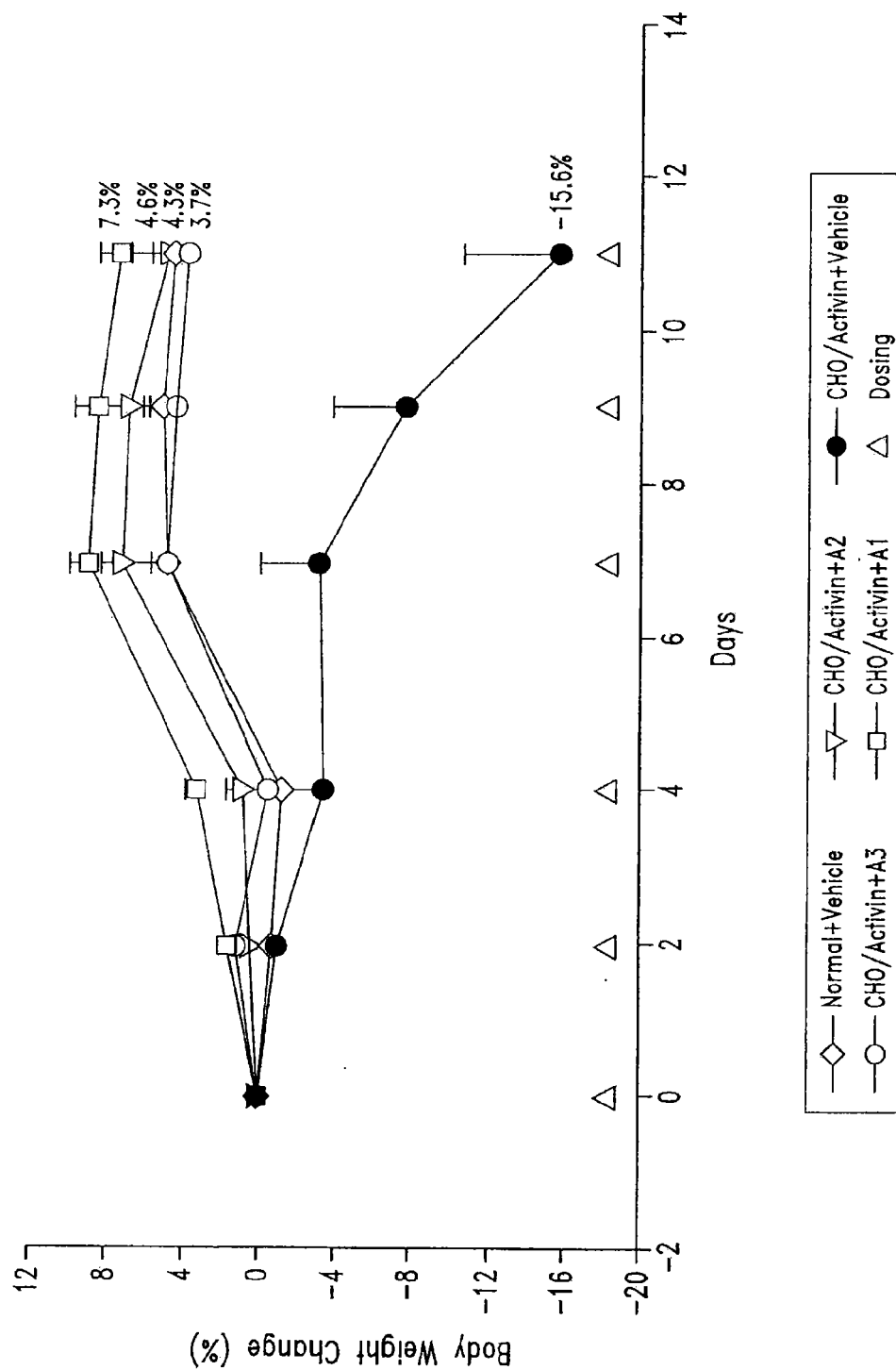


Fig. 3

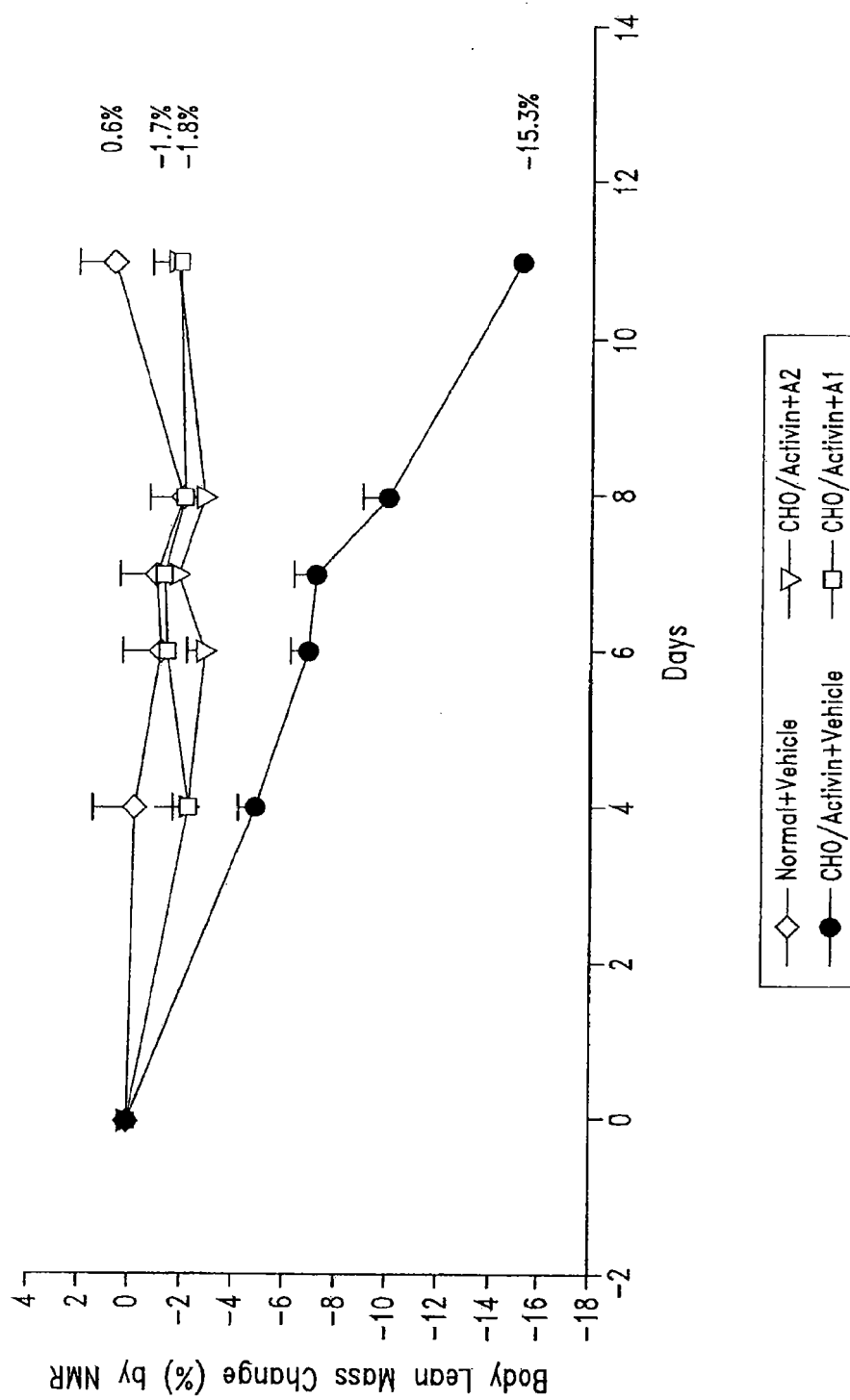


Fig. 4

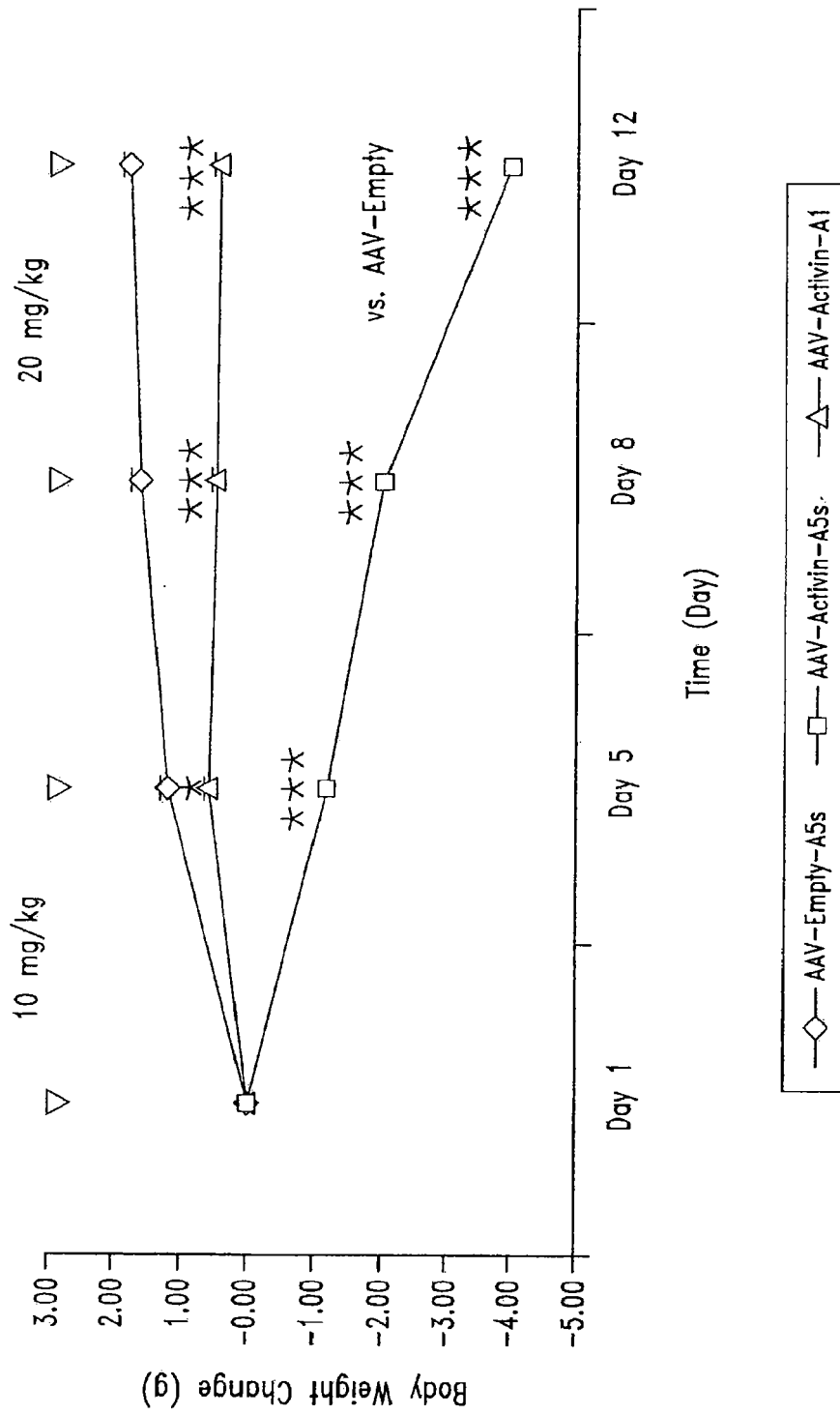


Fig. 5

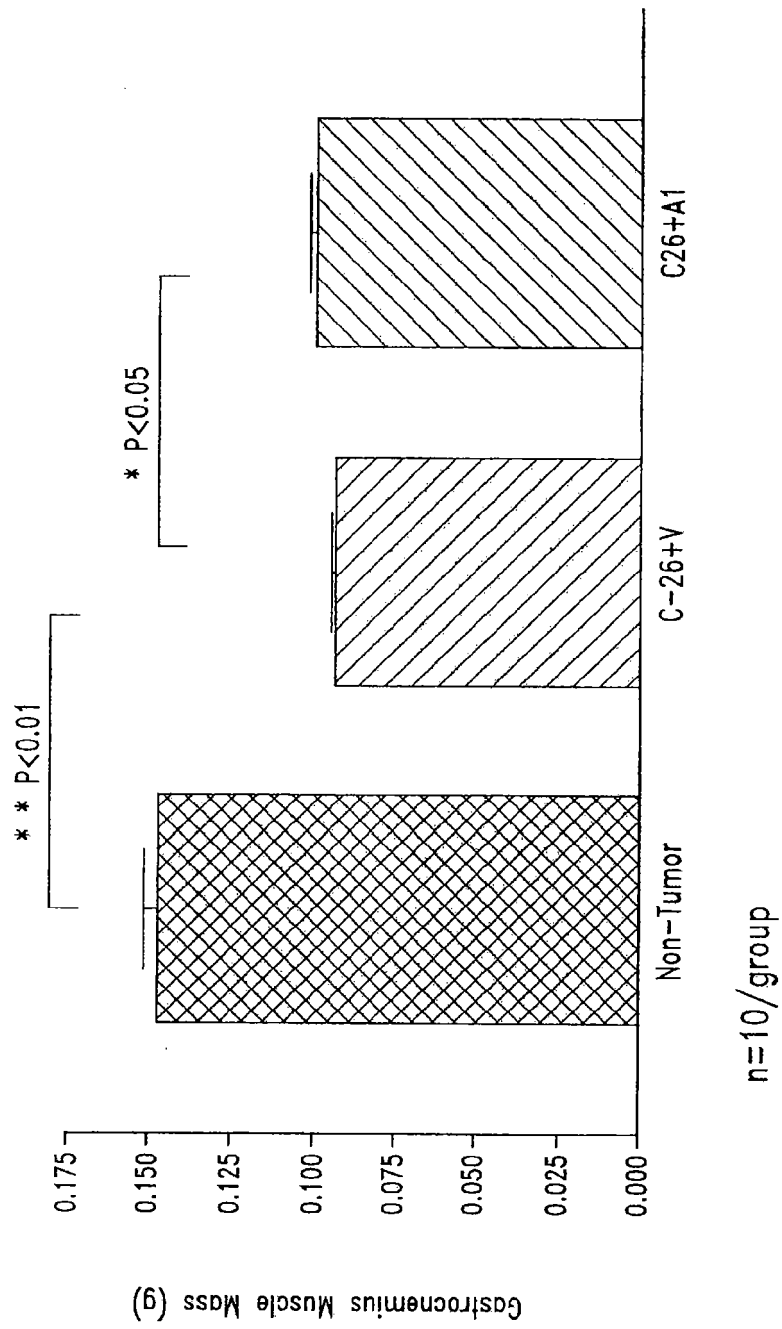


Fig. 6

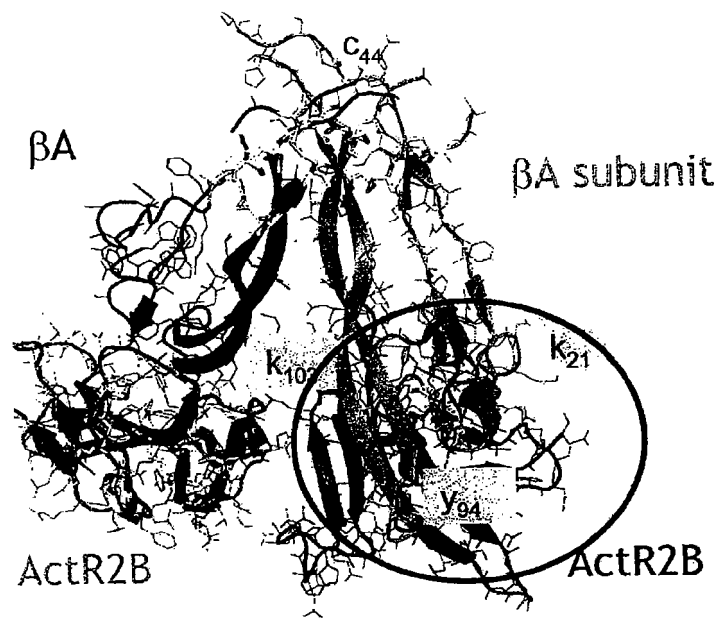


Fig. 7

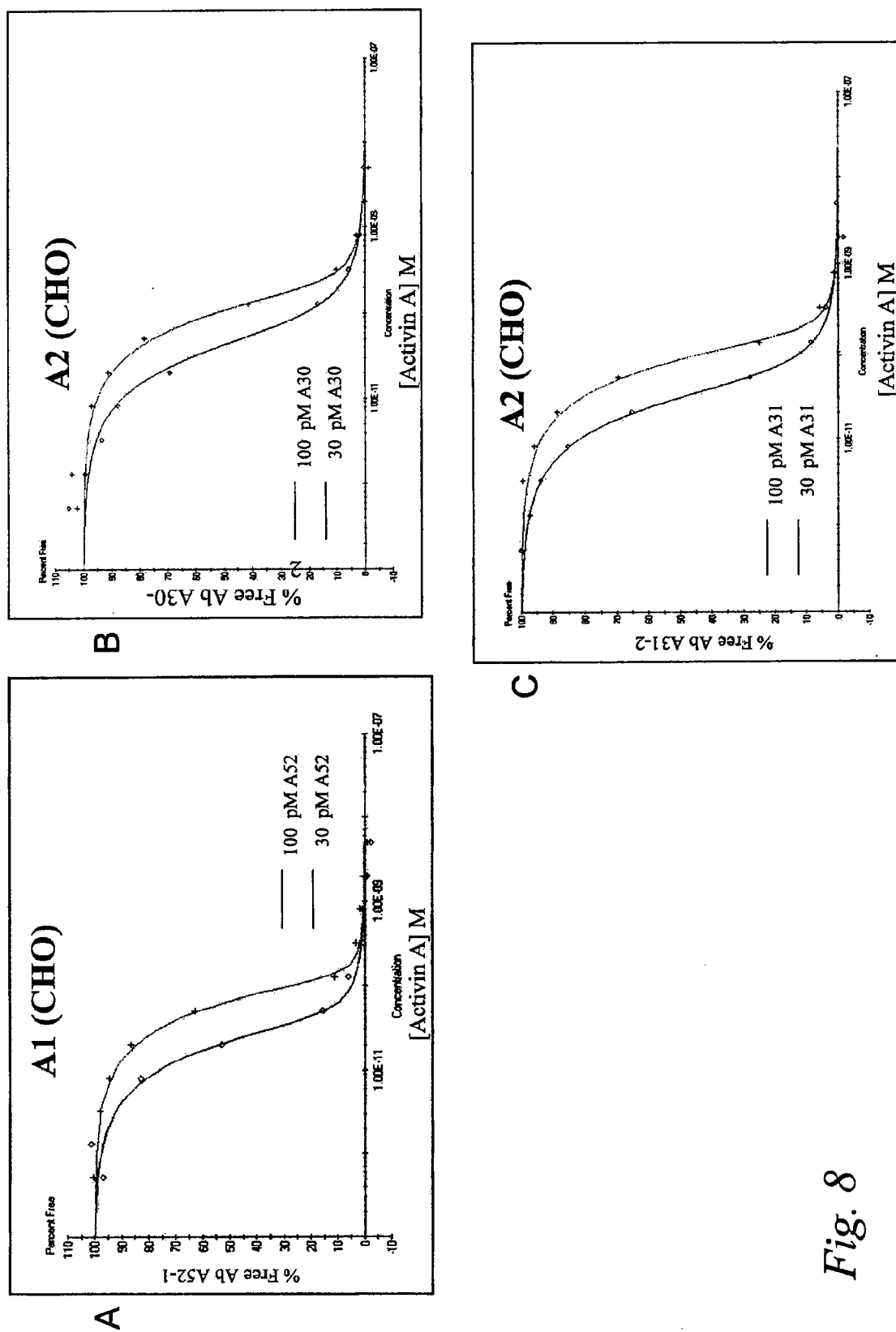
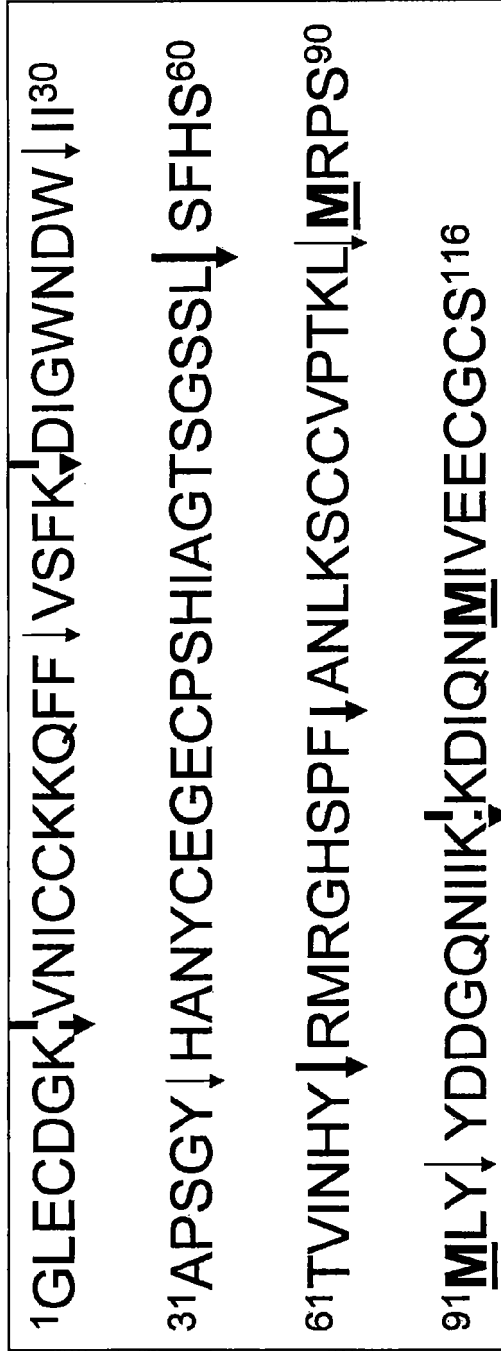


Fig. 8



Solid arrows – chymotrypsin cleavage

Broken arrows – LysC cleavage

M – putative CNBr cleavage

Bold arrows and residues were not protected by antibody binding.

Fig. 9

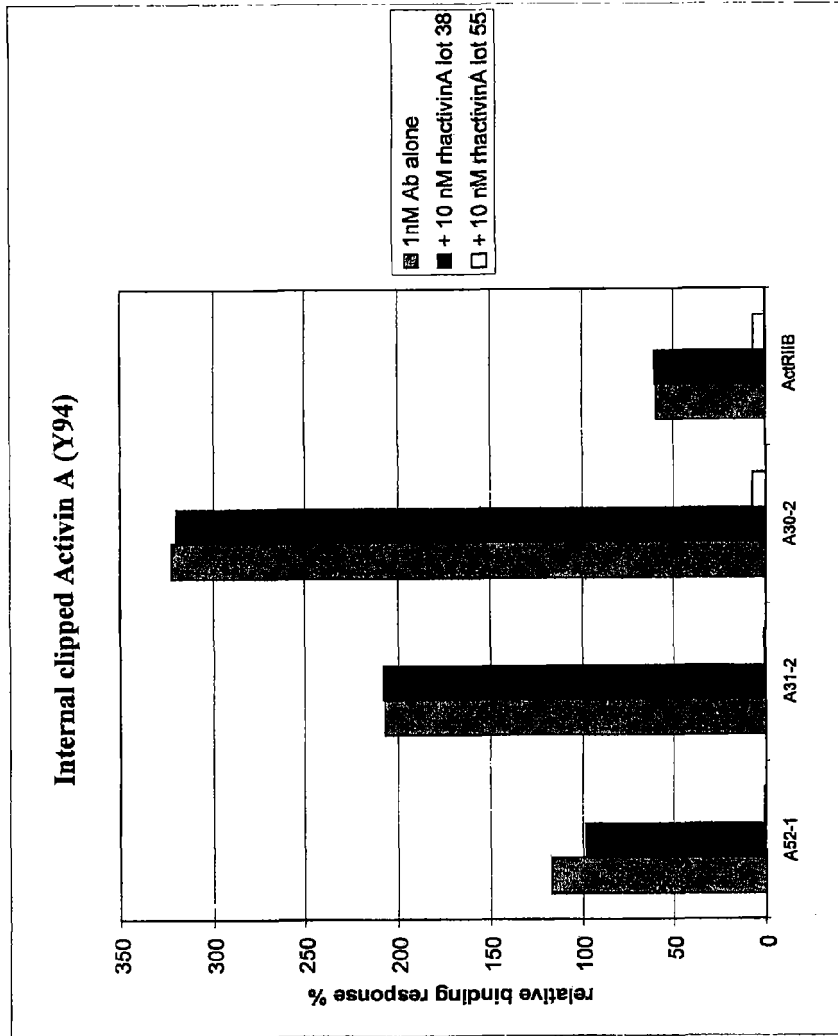


Fig. 10

2 nM rhActivinA + 20 nM Abs on immobilized Ab surfaces

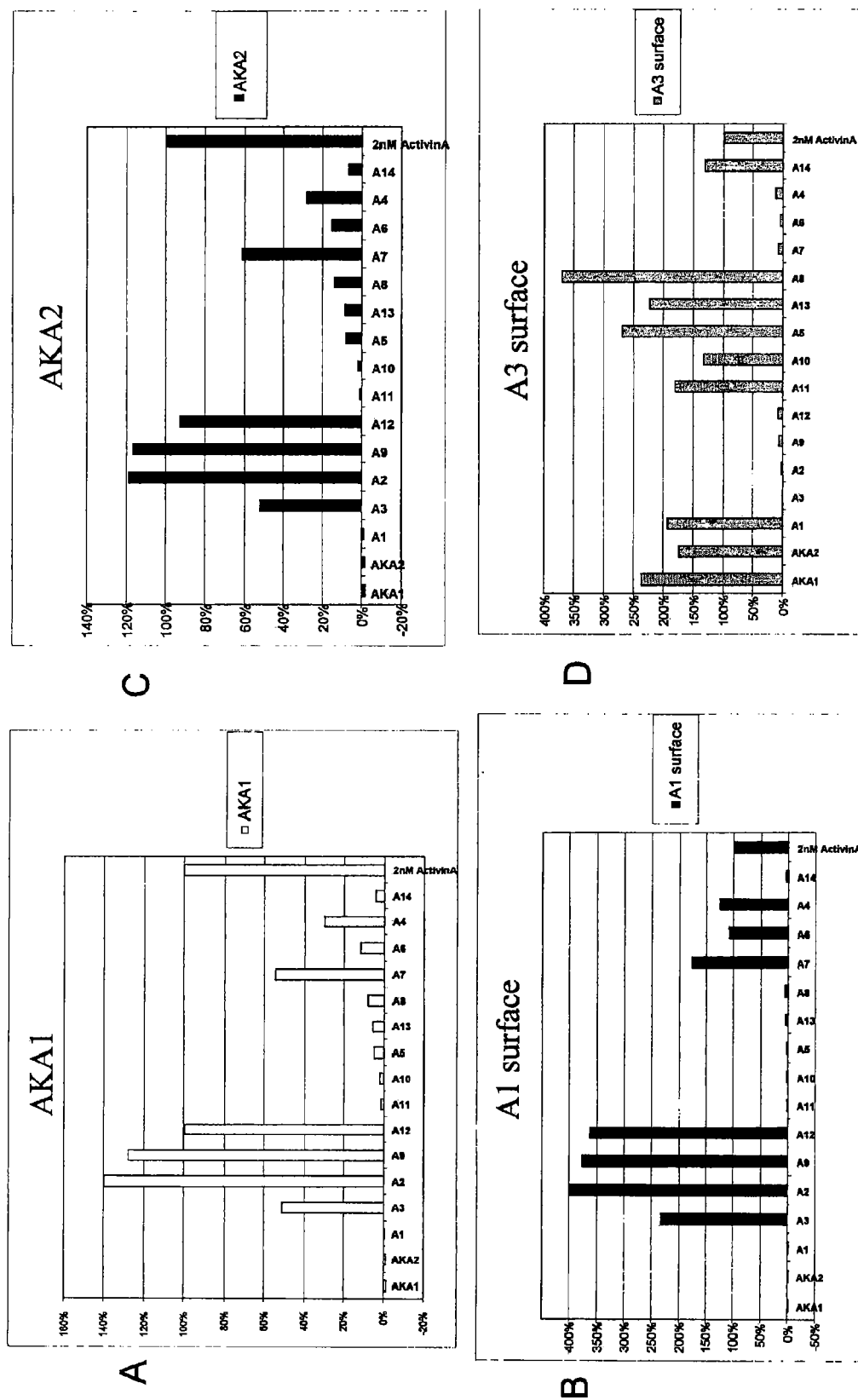


Fig. 11

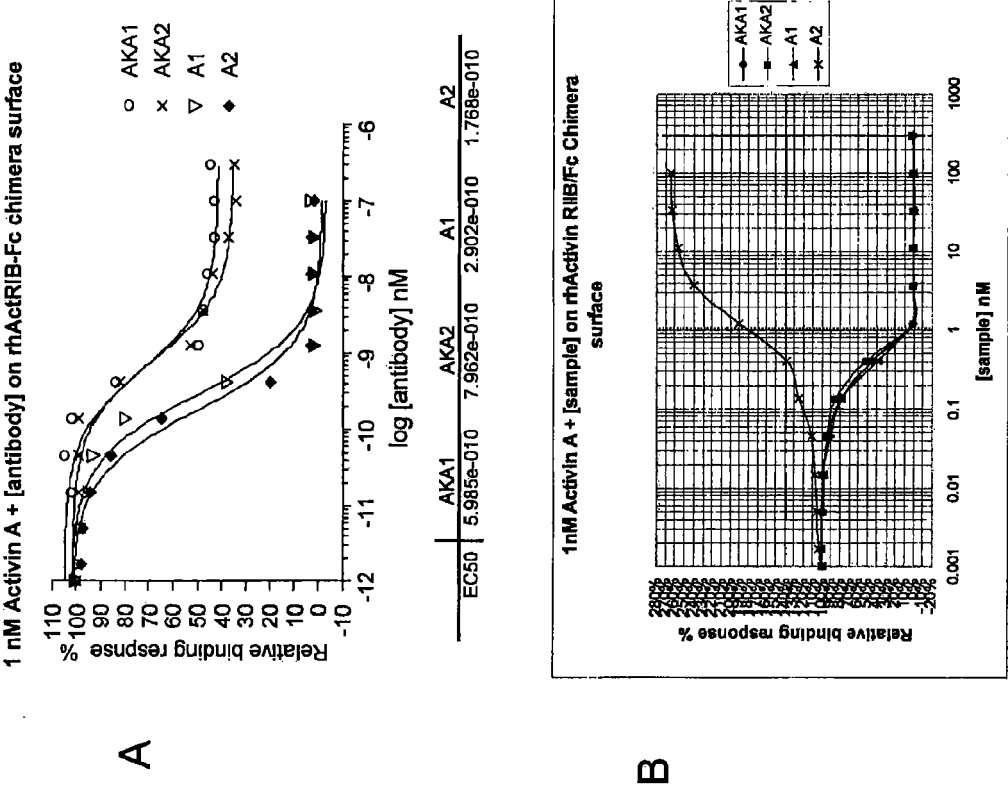


Fig. 12

Activin A/B Chimeras

	(1)	1	10	20	30	40	54
Activin A 13/39 B	(1)	GLECDGKVNICCRQ	QFFIDFRLIGW	NDWIIAPTGY	YGN	YCEGECPSH	IAGTSGS
Activin A 82/107 B	(1)	GLECDGKVNICCKK	QFFVSEFKDIG	WNDWIIAPSGY	HANYCEGECPSH	IAGTSGS	
Activin A	(1)	GLECDGKVNICCKK	QFFVSEFKDIG	WNDWIIAPSGY	HANYCEGECPSH	IAGTSGS	
							Section 2
	(55)	55	60	70	80	90	108
Activin A 13/39 B	(55)	SLSFHSTVINHYR	MRGHSPPFANL	KSCCVPTKLR	PM	SMLYYDDGQ	NIKKDIQNM
Activin A 82/107 B	(55)	SLSFHSTVINHYR	MRGHSPPFANL	KSCCIP	TKLSTMS	MLYFDDDEY	NIIVKRDVPM
Activin A	(55)	SLSFHSTVINHYR	MRGHSPPFANL	KSCCVPTKLR	PM	SMLYYDDGQ	NIKKDIQNM
							Section 3
	(109)	109	116				
Activin A 13/39 B	(109)	IVEECGCS					
Activin A 82/107 B	(109)	IVEECGCS					
Activin A	(109)	IVEECGCS					

Fig. 13

100 nM Antibodies block Activin A/B chimera on ActRIB-Fc Surface

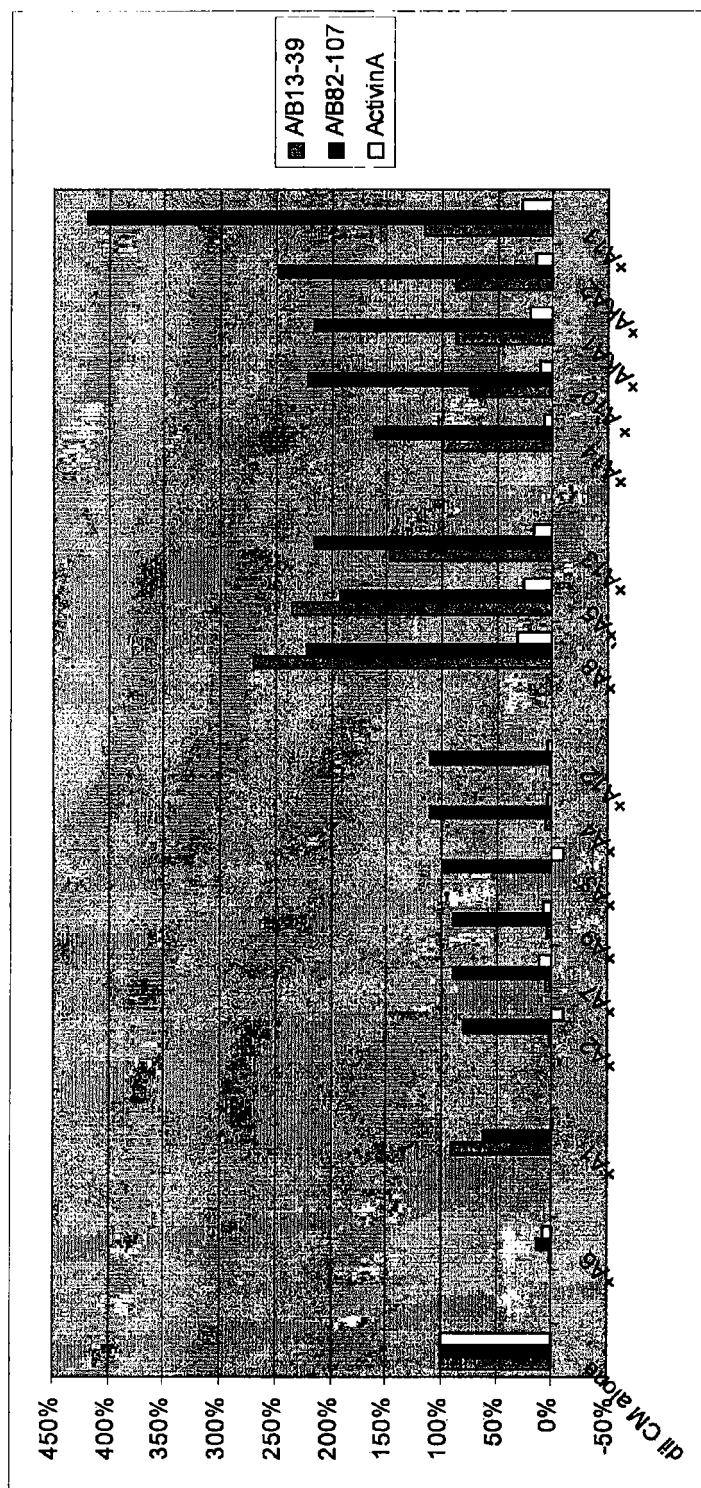


Fig. 14



EUROPEAN SEARCH REPORT

 Application Number
 EP 20 17 5809

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	DATABASE WPI Week 198933 Thomson Scientific, London, GB; AN 1989-237375 XP002471408, & JP 1 171495 A (AJINOMOTO KK) 6 July 1989 (1989-07-06) * abstract *	1-11	INV. C07K16/22 A61K39/395 C12N15/13 A61P5/06 A61P9/10 A61P35/00 A61P37/00 A61P43/00
A	----- RABINOVICI J ET AL: "LOCALIZATION AND REGULATION OF THE ACTIVIN-A DIMER IN HUMAN PLACENTAL CELLS", JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, vol. 75, no. 2, 1992, pages 571-576, XP002471406, ISSN: 0021-972X * the whole document *	1-11	A61P1/00 A61P3/04 A61P5/00 A61P7/00 A61P9/00 A61P19/02 A61P29/00
A	----- GROOME N ET AL: "PREPARATION OF MONOCLONAL ANTIBODIES TO THE BETA A SUBUNIT OF OVARIAN INHIBIN USING A SYNTHETIC PEPTIDE IMMUNOGEN", HYBRIDOMA, vol. 10, no. 2, 1991, pages 309-316, XP009096753, ISSN: 0272-457X * the whole document *	1-11	TECHNICAL FIELDS SEARCHED (IPC) C07K A61K
A	----- R&D SYSTEMS, INC , 12 October 2004 (2004-10-12), XP002471407, Retrieved from the Internet: URL:http://www.rndsystems.com/pdf/mab3381.pdf [retrieved on 2008-06-09] * the whole document * ----- -/--	1-11	
The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 6 August 2020	Examiner Bayer, Annette
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

EPO FORM 1503 03.82 (P04C01)



EUROPEAN SEARCH REPORT

Application Number
EP 20 17 5809

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	HARRISON C A ET AL: "Antagonists of activin signaling: mechanisms and potential biological applications", TRENDS IN ENDOCRINOLOGY AND METABOLISM, ELSEVIER SCIENCE PUBLISHING, NEW YORK, NY, US, vol. 16, no. 2, 1 March 2005 (2005-03-01), pages 73-78, XP027790070, ISSN: 1043-2760 [retrieved on 2005-03-01] * the whole document * -----	1-11	
			TECHNICAL FIELDS SEARCHED (IPC)
The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 6 August 2020	Examiner Bayer, Annette
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 20 17 5809

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